BETTER TOGETHER

Evaluating single and combined biomarkers of fermented food intake and their associations with cardiometabolic health

Katherine Jia Li

Propositions

1. Proper study design is the biggest challenge for large-scale metabolomics studies.
   (this thesis)

2. Consuming probiotic fermented foods provides greater health benefits than taking a probiotic supplement.
   (this thesis)

3. There are no scientific breakthroughs; scientific advancement from research is an incremental process.

4. Taking a philosophy course in university will help students become better writers.

5. Telling children to 'follow their passion' is bad career advice.

6. A beautiful presentation makes more impact on an audience than an accurate presentation.

Propositions belonging to the thesis, entitled

Better Together: Evaluating single and combined biomarkers of fermented food intake and their associations with cardiometabolic health

Katherine Jia Li
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Better Together:

Evaluating single and combined biomarkers of fermented food intake and their associations with cardiometabolic health

Katherine Jia Li

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CHAPTER 1

General introduction

Poor diets contribute to the increasing burden of non-communicable diseases (1). In 2017, over 11 million deaths were attributed to dietary risk factors, with the vast majority of deaths resulting from cardiometabolic diseases (CMDs) including cardiovascular disease (CVD) (~10 million) and type II diabetes (~339,000) (2). As such, defining diets and dietary patterns that mitigate CMD risk is of great public health importance. Recently, the consumption of fermented foods has emerged as an important dietary strategy for improving cardiometabolic health (3). Fermented foods have been present in the human diet for over 10,000 years (4, 5), but knowledge on whether their consumption benefits human health, and the molecular and microbiological mechanisms underpinning their purported health benefits, is relatively nascent. In this chapter, an outline is provided of the definitions of fermented foods, the types and qualities of fermented foods consumed in Europe, as well as the current state of the evidence between fermented foods and cardiometabolic health. Subsequently, the limitations in current self-report dietary assessment tools that contribute to the conflicting evidence between fermented foods and cardiometabolic health will be discussed, and the role of food intake biomarkers in providing a more objective measure of intake. Finally, the objectives of this thesis will be presented.

What constitutes a 'fermented food'?

For millennia, fermentation has been used an effective method of food preservation and alcohol production (6). Fermentation of raw agricultural products can improve their nutritional qualities as well as impart new aromas and tastes. However, the increase in the popularity of fermented foods over the past several decades has led to widespread misconceptions about what is required for a food to be considered 'fermented'. Several definitions of fermented foods have been proposed over the years. One of the earlier documented descriptions, by Steinkraus (7), captures the biological complexity and transformative nature of food fermentation: "Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids to non-toxic products with flavours, aromas and textures pleasant and attractive to the human consumer." However, this definition does not capture the intentional nature of food fermentation processes, and seemingly limits the transformative components in food to macronutrients. In 2021, the International Scientific Association for Probiotics and Prebiotics (ISAPP) provided a consensus statement on fermented foods, broadly defining fermented foods as: "Foods made through desired microbial growth and enzymatic conversions of food components" (8). Under this definition, fermented foods are those formed through a controlled process involving 'desired' microorganisms. Importantly, the broad reference to conversions of 'food components' suggests that the fermentation process could generate novel dietary compounds with distinct functional properties.

All fermented foods are procured via the actions of fermentative microorganisms. Microorganisms naturally present in the raw food matrix or the surrounding environment can initiate 'spontaneous' fermentations, such as during the fermentation of cocoa beans using indigenous yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) (9). However, large-scale industrial fermentations, which are becoming more commonplace, typically use starter cultures to ensure consistency in the end food product (10). Advances in food technology has also led to the application of alternative processing of foods that were traditionally fermented, resulting in non-fermented products, notably: pickled vegetables preserved in vinegar or brine, meat or fish preserved using salt, bread leavened using baking powder, and fresh cheeses curdled with vinegar or citric acid (**Table 1**).

Table 1. Classification of fermented foods
Fermented
Live microorganisms present
• Yoghurt • Sour cream • Kefir • Most cheeses • Miso • Natto • Tempeh • Non-heated fermented vegetables • Non-heated salami, pepperoni and other fermented sausages • Boza, bushera and other fermented cereals • Most kombuchas • Some beers
Live microorganisms absent
• Bread • Heat-treated or pasteurized fermented vegetables, sausage, soy sauce, vinegar and some kombuchas • Wine, most beers and distilled spirits • Coffee and chocolate beans (after roasting)
Not fermented
• Chemically leavened bread • Fresh sausage • Vegetables pickled in brine and/or vinegar • Chemically produced soy sauce • Salted or cured processed meats and fish

Adapted from Marco *et al.* (8).

While the consumption of fermented foods tends to be synonymous with consuming a 'dose' of live microorganisms, this is not always the case. The presence of live microorganisms in the ready-to-consume product is dependent on several factors: the phase of the fermentation (*e.g.*, fermentation of cocoa beans in the food preparation phase *versus* fermentation of yoghurt in the final product phase), whether the fermented food is heat-treated or if the microorganisms are intentionally removed (*e.g.*, filtration of wine), as well as personal food preferences (*e.g.*, cooked sauerkraut consumed in The Netherlands). These factors create a key delineation (*i.e.,* presence/absence of microorganisms) for the classification of different types of fermented foods with relevance to their health impacts (**Table 1**).

Fermented foods as a source of live microorganisms

In Western societies, the resurgent interest in the consumption of fermented foods can be credited to the explosion of research into the human microbiome (11, 12). Several studies have demonstrated that diet influences the structure and function of the gut microbiota (13, 14). It is believed that the consumption of fermented foods containing probiotics – "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (15) – is an effective way to introduce potentially beneficial microorganisms to the intestinal tract and help manage a wide range of disorders associated with gut microbial dysbiosis. These include both intestinal disorders, such as behaviour and brain disorders, inflammatory bowel disease, irritable bowel syndrome, and coeliac disease, as well as extra-intestinal disorders, including allergy, asthma, obesity, metabolic syndrome, and CVD (16, 17).

The diversity of microorganisms found in fermented foods produced globally, as well as their functional properties, have been the subject of several comprehensive reviews (18, 19). Primarily, these include gram-positive (particularly LAB) and gram-negative bacteria, filamentous molds, and enzyme- and alcohol-producing yeasts (18). These microorganisms and their enzymes have varied functional roles, such as acting as antimicrobial agents (20), antioxidants (21), and fibrinolytic agents (19, 22). Recent advances in (meta)genomic sequencing are further expanding our understanding of the microbial diversity and functional potential of fermented foods, in particular bacterial and fungal species that have been less well-characterized due to difficulties in culturing these species (12, 23).

Another important aspect affecting the health impact of fermented foods is the amount of live microorganisms provided by the consumption of fermented foods. In a review by Rezac *et al.* (24), many fermented foods (cheese, yoghurt, sausages, vegetables, cereals, sour beer, kombucha, fermented fish, and tempeh) were found to contain 10^{5-7} colony forming units (CFU) of LAB/(mL or g), with cultured dairy products containing up to 10^{9} CFU/(mL or g). However, considerable variation was observed based on geographical region and sampling time, in addition to the manufacturing, processing, and storage conditions

(24). Although guidelines are lacking for the minimum dose of live microorganisms that should be consumed, the European Union (EU) health claim for yoghurt and "improved lactose tolerance" stipulates that at least 10^8 CFU of live starter microorganisms per gram of yoghurt (25). Further, Derrien *et al.* (26) predicted that ingesting a dose of 10^{10} ingested bacterial cells would be sufficient to drastically shift the composition of the gut microbiota and impact the immune and neuroendocrine responses of the host.

Fermented foods as a source of fermentation-derived metabolites

While not all fermented foods contain live microorganisms at the time of consumption, microbial activity during fermentation can still produce bioactive metabolites that could be beneficial to human health (27). The main fermentation processes can be grouped by the primary metabolites of fermenting microorganisms: alcohol and carbon dioxide produced by yeasts, acetic acid produced by AAB, lactic acid produced by LAB, ammonia and fatty acids produced by *Bacilli* and molds, and propionic acid produced by propionic acid bacteria (3). These metabolites parallel the end-products of the fermentation of undigested carbohydrate and protein by the gut microbiota, some of which (*e.g.*, organic acids) have been positively associated with host gastrointestinal and immune health, lipid and protein metabolism, and appetite control (28).

Additionally, various secondary 'bioactive' metabolites produced during fermentation are receiving increasing scientific interest for their functional properties. The types of metabolites produced depends on both the substrate and type of fermentation. For example, fermentation of milk results in the production of αs1- and β-casein peptide fragments from milk casein, which have been detected in several varieties of cheese (29). These bioactive peptides have angiotensin-converting enzyme (ACE) inhibitory activity and have also been reported to modulate opioid receptors in the gut epithelium (29). On the other hand, fermentation of multiple foods (*e.g.*, cheese, sauerkraut) via a common fermentation pathway using LAB generates phenyllactic acids which help with food preservation as well as serve a physiological role of immune modulation (30, 31).

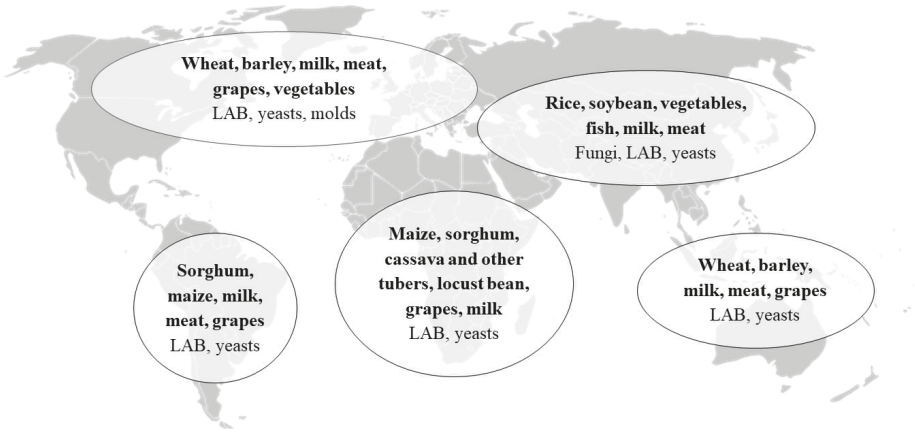
Fermentation to enhance the nutritional quality of foods

In addition to introducing novel fermentation-derived metabolites and directly influencing nutrients, fermentation can also enhance the nutritional composition of the final food product or improve nutrient bioavailability. Various fermented foods have been shown to have enhanced nutritional attributes compared to their non-fermented counterparts (32-35). For example, it has been observed that levels of flavonoids, anthocyanins, and triterpenoids progressively increased during the fermentation of raw radishes, beets, and peppers (33). Fermentation of milk into cheese and yoghurt has been shown to increase the levels of free amino acids detected in plasma, including α-amino butyric acid, alanine, asparagine, cysteine, glycine, glutamine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine (34, 35). Further, fermentation can also inactivate toxic dietary components and degrade anti-nutritional factors (36). Phytates present in cereals, legumes, and tubers are decreased during fermentation as a result of the activity of microbial phytases (36). The combination of fermentation and cooking also inactivates lectins from legumes that hinder nutrient absorption from the gastrointestinal tract (36).

Fermented foods consumed in global and European diets

Over 5,000 types of fermented foods and beverages have been estimated to exist worldwide, contributing to 5-40% of the human diet (8, 37, 38). Every region and culture produces distinct fermented foods based largely on accessibility to different raw materials (**Figure 1**). The specific types and qualities of fermented foods consumed in different cultures around the world have been the subject of several comprehensive reviews (18, 39, 40).

Figure 1. Predominant fermented food substrates and microorganisms in regions around the world. Adapted from Tamang *et al.* (40). LAB, lactic acid bacteria.



In Europe, fermented foods were traditionally derived from fermentation of milk, wheat, barley, meat, grapes, and vegetables. Multiple varieties of cheeses, wine, beer, and dried/salted meats (including fermented meats) are designated as goods with "specific geographical origin" in the EU and have distinct historical and cultural significance (41). Several other fermented foods, such as coffee and chocolate, have origins in other cultures but have been normalized in European diets from ancient trade routes or colonization. It is expected that the varieties of fermented foods in European diets will continue to expand with globalization and consumer tastes for healthy and exotic flavours. A non-exhaustive overview of the types of fermented foods commonly consumed in modern European diets, as well as their raw materials and fermenting microorganisms, is provided in **Table 2**. It should be noted that although spirits (*e.g.*, brandy, gin, whisky) are also technically considered to be fermented beverages, they were not further evaluated in this thesis, as their high alcohol content dominates the presence of other metabolites of fermentation that are interesting from a nutritional point of view.

Alongside these fermented foods, dairy products as a whole (which comprise both fermented and non-fermented dairy foods), make a significant nutritional contribution to European diets. For decades, dairy foods have been relied upon as a source of different macronutrients and micronutrients, including calcium, vitamins, protein, essential and non-essential fatty acids (42). A wide variety of dairy foods exist in the European food supply: yoghurt, cheese, fermented milks, crème fraiche, buttermilk, and quark (typically fermented), as well as milk, fresh cheeses, cream, ice cream, and butter (typically non-fermented). The levels of dairy nutrients and their associated health implications have been extensively studied, and formed the basis of conventional dietary recommendations that promote dairy to reduce nutrient deficiencies and prevent over-nutrition (1, 42, 43). However, dairy foods are more than a 'sum of their parts', and recent recognition of the complexity of diet-disease risk pathways has highlighted the importance of examining whole foods – taking into consideration the food matrix, nutrients, other ingredients, and different processing methods (*e.g.*, fermentation) – for chronic disease prevention (43).

Table 2. Substrates and microorganisms of predominant fermented foods consumed in European diets

Fermented food	Substrate[a]	Fermenting Microorganisms	Live microorganisms at time of consumption	Reference
Cheese (Afuega'l Pitu, Armada, Asiago, blue cheese, Brie, Burgos, Cabrales, Camembert, Cheddar, Comte, Danbo, Edam, Feta, Fontina, Galotyri, Gorgonzola, Gouda, Gubeen, Grana Padano, Havarti, Livarot, Limburger, Manchego, Monterey Jack, Mozzarella, Muenster, Parmesan, Puzzone di Moena, Pecorino Romano, Provolone, Stilton, Swiss, Swiss Gruyere, Tilsit)	Milk (bovine)	• **LAB:** *Lb. paracasei, Lb. rhamnosus, Lb. delbrueckii* subsp. *Bulgaricus, Lb. delbrueckii* subsp. *delbrueckii, Lb. delbrueckii* subsp. *lactis, Lb. helveticus, Lb. casei, Lb. plantarum, Lb. salivarius, Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis, Leuc.* spp., *Ent.* spp. (*Ent. durans, Ent. faecium*), *Strep. thermophilus* • **Other gram-positive bacteria:** *Staph.* spp., *Brevibacterium linens, Propionibacterium freudenreichii* • **Fungi:** *Debaryomyces hansenii, Geotrichum candidum, P. camemberti, P. roqueforti*	Yes (most)	(18, 24)
Yoghurt	Milk (bovine)	• **LAB:** *S. thermophilus, Lb. bulgaricus, Lb. acidophilus, Lb. casei, Lb. rhamnosus, Lb. delbrueckii* subsp. *bulgaricus , Lb. gasseri, Lb. johnsonii* • **Other gram-positive bacteria:** *B. lactis, B. bifidum*	Yes	(18, 24)
Buttermilk	Milk (bovine)	• **LAB:** *Lactococci, Lactobacilli*	Yes	(6, 24)
Bread (white, wholegrain, sourdough)	Barley, rye, wheat	• **Yeasts:** *Sacc. cerevisiae, C. humili, Issatchenkia orientalis* • **LAB (sourdough only):** *Lb. sanfranciscensis, Lb. alimentarius, Lb. buchneri, Lb. casei, Lb. delbrueckii, Lb. fructivorans, Lb. plantarum, Lb. reuteri, Lb. johnsonii*	No	(18)
Dried fermented sausage (Salami, Salsiccia, Soppressata, Alheiras, Botillo, Chorizo, Salchicón, Pepperoni)	Pork or beef	• **LAB:** *Lb. plantarum, Lb. paraplantarum, Lb. brevis, Lb. rhamnosus, Lb. sakei, Lb. zeae, Lb. paracasei, Ent. faecalis, Ent. faecium, Leuc. mesenteroides, Ped. pentosaceus, Ped. acidilactici, W. cibaria, W. viridescens, Lb. sake, Lb. curvatus, Lb. plantarum* • **Other gram-positive bacteria:** *Micrococcus* spp., *Staph.* spp. • **Gram-negative bacteria:** *Enterobacteriaceae* • **Yeasts and molds**	Yes	(24)
Sauerkraut	Cabbage	• **LAB:** *Leuc. mesenteroides, Ped. pentosaceus; Lb. brevis, Lb. plantarum, Lb. sakei*	Yes	(18, 24)
Fermented cucumbers	Cucumbers	• **LAB:** *Leuc. mesenteroides, Ped. cerevisiae, Ped. acidilactici, Lb. plantarum, Lb. brevis*	Yes	(18)
Fermented olives	Olives	• **LAB:** *Leuc. mesenteroides, Ped. pentosaceus; Lb. plantarum Lb. pentosus/Lb. plantarum, Lb. paracollinoides, Lb. vaccinostercus, Lb. suebicus, Ped.* sp. • **Other gram-positive bacteria:** *Gordonia* sp. • **Gram-negative bacteria:** *Pseudomonas* sp., *Sphingomonas* sp., *Sphingobium* sp., *Sphingopyxis* sp., *Thalassomonas agarivorans,* • **Yeasts:** *C. cf. apicola, Pic.* sp., *Pic. Manshurica, Pic. galeiformis, Sacc. cerevisiae* • **Archaea:** *Halorubrum orientalis, Halosarcina pallid*	Yes	(18)
Coffee	Coffee cherries	• **LAB:** *Lc. Lactis, Leuc. Mesenteroides, Lb. plantarum, Lb. brevis* • **Other gram-positive bacteria:** *Bc. cereus, Bc. megaterium, Bc. subtilis, Bc. Macerans* • **Gram-negative bacteria:** *Serrati* sp., *Enterobacter agglomerans, Klebsiella pneumonia, Erwinia herbicola, Acinetobacter* sp., *Escherichia coli*	No	(44)

		• **Yeasts:** *Pic. anomala, Torulaspora delbrueckii, Rhodotorula mucilaginosa, C. ernobii, C. carpophila, Saccharomyces* sp., *Pic. caribbica, C. membranifaciens, Arxula* sp., *Hanseniaspora uvarum, Kluyveromyces* sp., *Kloeckera* sp., *C. xestobii*		
Chocolate	Cocoa pods	• **AAB:** *A. pasteurlanus, A. senegalensis* • **LAB:** *Lb. fermentum, Lb. ghanensis, Lb. brevis, Leuc. mesenteroides, Leuc. pseudomesenteroides, W. ghanensis, Lb. cacaonum, Lb. fabifermentans, W. fabaria, Fructobacillus pseudoficulneus, Lb. plantarum* • **Gram-negative bacteria:** *Enterobacteria, Tatumella ptyseos, Tatumella citrea* • **Yeasts:** *Sacc. cerevisiae, Kluyveromyces, Hanseniaspora uvarum, Hanseniaspora quilliermundii, Issatchenkia orientalis (C. krusei), Pic. membranifaciens*	No	(18, 45)
Wine	Grapes	• **Yeasts:** *Sacc. cerevisiae, C. colliculosa, C. stellata, Hanseniaspora uvarum, Kloeckera apiculata, Kl. thermotolerans, Torulaspora delbrueckii, Metschnikowia pulcherrima, Candida* sp. *and Cladosporium* sp.	No	(18)
Beer	Barley, hops	• **Yeasts:** *Sacc. cerevisiae, Sacc. carlsbergensis, Sacc. pastorianus*	No	(18)

A., Acetobacter; AAB, acetic acid bacteria; *B., Bifidobacterium; Bc., Bacillus; C., Candida*; LAB, lactic acid bacteria; *Ent., Enterococcus; Lb, Lactobacillus; Lc, Lactococcus; Leuc. Leuconostoc; P., Penicillium; Ped., Pediococcus; Pic., Pichia; S, Streptococcus; Sacc., Saccharomyces; Staph., Staphylococcus; W., Weissela*.

[a] Most common substrates listed, other substrates can also be used.

The evidence between fermented foods and cardiometabolic health

Proposed mechanisms between the consumption of fermented foods and health

Several mechanisms have been proposed that support a role of fermented foods in promoting (cardiometabolic) health. As mentioned previously, fermented foods containing live microorganisms at the time of consumption may provide a source of probiotics, which can modulate both the composition and function of the host's gut microbiota (26). Changes in gut microbial composition could enhance the integrity of the intestinal barrier and reduce low-grade inflammation associated with endotoxemia, which is speculated to be a mediator of obesity-related diseases (46). In animal models, consumption of dairy products with probiotics demonstrated greater cardiometabolic health benefits compared to consumption of dairy products without probiotics. In one such study, C57BL/6 mice on high-fat diets given kefir (a fermented dairy product) had reduced weight gain, hepatic steatosis, and low-density lipoprotein (LDL)-cholesterol levels compared to mice given milk (47). Mice given kefir also had higher levels of *Lactobacillus, Lactococcus*, total yeast, and *Candida* in the gut, which was strongly correlated with upregulated expression of fatty acid oxidation genes (*AOX*, *PPAR-α*) in both hepatic and adipose tissues. Reduced plasma levels of the pro-inflammatory cytokine IL-6 and down-regulation of the inflammation gene *MCP1* in adipose tissue was also observed. Evidence from several human trials also support a promising role for certain probiotic strains (primarily *Lactobacillus*) on weight maintenance, adiposity, obesity, and cholesterol levels, although further evidence is needed for their clinical relevance (48-52). Moreover, consumption of probiotics seems to modulate the function of the gut microbiota by increasing the production of short-chain fatty acids that impact energy homeostasis, obesity, and insulin resistance (53, 54). Given that there is a clear overlap between fermentation products and microbial activities in fermented foods and the gut microbiota, the literature on the health impact of the gut microbiota feeds back into the potential health benefits of fermented foods.

On the other hand, fermented foods that do not contain live microorganisms at the time of consumption can still be a source of postbiotics, defined as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (55). The mechanisms of the non-viable microorganisms have been postulated to be similar to probiotics, such as helping to modulate the gut microbiota and enhance epithelial barrier function, as well as modulating host immune, metabolic, and signaling responses (55). In mouse models, consumption of heat-inactivated postbiotic preparation consisting of *Limosilactobacillus fermentum* and *Lactobacillus delbrueckii* resulted in altered gut microbiota composition and intestinal structure, indicating that the inactivated microorganisms maintain biological activity (56).

Importantly, both fermented foods containing live probiotics or inactive postbiotics contain end-products of fermentation (*i.e.*, metabolites derived from or enhanced by the fermentation process), which could modulate multiple metabolic signaling pathways to improve overall cardiometabolic health. Many fermented foods are produced using LAB, which generates lactic acid, short-chain fatty acids, bioactive peptides, and polyamines with potential effects on cardiovascular, immune and metabolic health (55, 57). Fermenting bacteria can also influence cardiometabolic health by improving nutrient bioavailability, such as the bacterial production of vitamin K2 from vitamin K1, leading to a more potent activation of vitamin K-dependent proteins that affect multiple metabolic pathways (42). Other compounds may be present in fermented foods due to the presence of specific bacteria. For example, conjugated linoleic acid (CLA) that is associated with improved energy homeostasis (58) and which is already present in non-fermented dairy, may be elevated in fermented dairy foods due to the action of LAB or *Bifidobacteria* strains on linoleic acid (59).

Clinical evidence for fermented dairy and cardiometabolic health

Due to the nutritional importance of fermented and non-fermented dairy foods in European diets, a number of epidemiological and dietary intervention studies have investigated the impact of their consumption on CMDs and associated risk factors. Numerous studies have examined the cardio-protective effects of fermented dairy foods, both in the context of and separately to total dairy intake (60, 61). One example is the standardized analysis of the association between dairy intake and CVD risk in the Prospective Urban Rural Epidemiology (PURE) study, where dietary data on dairy consumption was assimilated for 136,384 individuals from 21 countries representing five continents (60). Overall, higher intake of dairy foods (> 2 servings/d) compared to no intake was associated with significantly lower risks of composite CVD events (combined mortality and major CVD events) (hazard ratio (HR) 0.84, 95% CI 0.75-0.94), cardiovascular mortality (HR 0.77, 0.58-1.01), major CVD events (HR 0.78, 0.67-0.90) and stroke (0.66, 0.53-0.82). Lower risks of composite CVD events were also observed for yoghurt (HR 0.86, 0.75-0.99) and milk (HR 0.90, 95% CI 0.82-0.99) intake, while neither cheese nor butter were associated with the clinical outcomes assessed.

In contrast, the findings of the Northern Sweden Health and Disease Study (n = 108,065) associated higher intakes of milk with an increased risk of myocardial infarction (HR 1.17, 95% CI 1.03-1.34) and type II diabetes (HR 1.23, 95% CI 1.10-1.37) in men but not women (61). Higher intakes of fermented milk, butter and cheese, however, were not significantly associated with these CVD-related outcomes. A stratification of the analysis by fat content surprisingly suggested that lower fat dairy products were associated with increased risk of the CVD-related outcomes assessed, but this may be attributed to other factors (*i.e.*, compensation of calories from dairy with that of other food groups, consumption of other foods in the diet in addition to low-fat dairy, or to possible cardio-protective effects of dairy fats).

Several other studies have also examined effects of fermented dairy intake on individual CMD risk factors, including blood lipids (62-66), hypertension (67-71), body mass index and obesity (72-77), type II diabetes, glycemia and insulin homeostasis (61, 75, 78, 79), also with conflicting findings. In a review of 16 meta-analyses by Gille *et al.* (80), weakly beneficial albeit inconsistent links were found between the consumption of dairy and fermented dairy products and several CMD risk factors (**Table 3**). The strongest evidence in the review was observed between yoghurt on risk factors of type II diabetes (80).

Table 3. Summary of the systematic reviews and meta-analyses between dairy foods and various cardiometabolic disease risk parameters[a]

	Total Dairy	Milk	Cheese	Yoghurt
Prospective studies				
CVD	○	●	○	○
CAD/CHD	○	○	○	○
Stroke	●	○	●	○
Hypertension	●	●	○	○
Metabolic synrome	●	●	●	●
Type II diabetes	●	○	●	●
Intervention studies				
LDL-cholesterol	○	○	○	○
HDL-cholesterol	○	●	●	○
Fasting triglycerides	○	●	○	○
Postprandial triglycerides	●	○	○	●
LDL size	●	○	●	●
Apolipoprotein B	●	○	○	●
Non-HDL cholesterol	●	●	●	●
Cholesterol ratios	●	○	○	●
Inflammation	○	○	●	○
Insulin resistance	●	○	○	○
Blood pressure	○	○	●	○
Vascular function	○	○	●	○

CAD, coronary arterial disease; CHD, coronary heart disease; CVD, cardivascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] Adapted from Gille *et al.* (80). Colors represent the overall findings of the studies: favourable (●), neutral or no effect (○), uncertain or undetermined (●). No adverse associations were observed.

Clinical evidence for other fermented foods and cardiometabolic health

Associations between bacterial fermented food intake and mortality from all causes, total cancer, and CVD were examined in 34,409 individuals from the EPIC-NL cohort (81). The fermented foods evaluated consisted mainly of fermented dairy foods (yogurt, buttermilk and quark, excluding cheese) (78%), and cheese (16%), but also fermented meat (dried sausage), vegetables (sauerkraut, pickles, olives), soya (tempeh) and vinegar. However, fermented foods made using yeast as the main starter culture (bread, wine, beer, alcoholic drinks) or by endogenous enzymes/microorganisms (cocoa, coffee, tea) were intentionally excluded. Higher total fermented food intake was not found to be associated with mortality due to all causes (HR 1.00, 95% CI 0.88-1.13), cancer (HR 1.02, 95% CI 0.86-1.21) or CVD (HR 1.04, 95% CI 0.83-1.30). Out of the fermented food subgroups, only cheese was moderately inversely associated with CVD mortality, particularly from stroke (HR 0.59, 95% CI 0.38-0.92).

The impact of several other fermented foods consumed in European diets on cardiometabolic health have also been the subject of comprehensive reviews and meta-analyses. Previous meta-analyses found no associations between coffee consumption and coronary heart disease (CHD) risk (82, 83), while an updated systematic review and meta-analysis of prospective cohort studies found an inverse association between moderate habitual coffee consumption and CVD risk (84). The relationships were non-linear, with the lowest risk reported at 3 to 5 cups of coffee per day. Here, genetic polymorphisms may be an important consideration to further interpret the heterogeneous impacts of coffee consumption on CVD-related outcomes (85).

Similarly, several meta-analyses have shown that low to moderate intake of red wine (*e.g.*, 270 mL/day) could have a protective effect against CHD, while extremely high intakes had deleterious effects (86-88). A review of prospective studies indicates that moderate beer consumption of up to 16 g alcohol/day (1 drink/day) for women and 28 g/day (1-2 drinks/day) for men may also have a protective effect against CVD and mortality compared to non-alcohol or occasional drinkers (89). However, the overall impact on different risk parameters remain equivocal. A recent meta-analysis of controlled clinical trials of beer intake found that beer drinkers had elevated total cholesterol, high-density lipoprotein (HDL)-cholesterol and apolipoprotein A1, and flow mediated dilation compared to non-beer drinkers, but no differences in LDL-cholesterol, triglycerides, blood pressure, or other biochemical markers of inflammation (90).

For chocolate, multiple meta-analyses have found that consumption of chocolate was inversely associated with CHD, stroke, and type II diabetes; however, effective categories or levels of intake differed among the studies and need to be further clarified, and several aspects of study quality needs to be improved (91-94). Aside from these fermented foods, few well-designed, randomized controlled trials or prospective studies have examined the health impacts of other fermented food products consumed in European or global diets. Presumably, since the types of fermented foods consumed varies greatly across the world, consumption of different microorganisms (and their products) that may have different health impacts as well.

Limitations of the current evidence

While a plethora of studies have examined associations between the intake of certain fermented foods (coffee, wine, beer, and cocoa) and cardiometabolic health outcomes, closer examination of the methods of these studies reveals limitations and inaccuracies which could obscure the interpretations of the results. In many studies, non-fermented foods have been misclassified as fermented food products. For example, in an association study between fermented dairy intake, diet quality, and cardiometabolic profile, Mena-Sánchez *et al.* (95) defined fermented dairy products as “low-fat yogurt, whole-fat yogurt, and all types of cheese (petit Swiss; ricotta; fresh cheese; cottage; and semi-cured and cured cheeses such as Cheddar, Manchego, and Emmental)”. Similarly, Kostinen *et al.* (96) included cottage cheese under total fermented dairy products when examining associations between fermented and non-fermented dairy products and risk of coronary heart disease. Ricotta, cottage cheese, and fresh cheeses are produced by curdling of milk with acid, and are thus

generally not considered to be fermented. In light of the recently clarified definitions of fermented foods, a more critical classification scheme is thus required to correctly classify fermented foods prior to examining associations between their intake and health outcomes. Furthermore, almost all studies to date have relied on self-report measures to assess the intake of fermented foods (81, 95, 96). The subjective nature of these tools can result in inaccurate estimates of the levels of fermented food intake. The characteristics and limitations of self-report dietary assessment tools are described in detail below, as well as an opportunity to improve the accuracy of assessing fermented food intake through more objective measures.

Methods for measuring dietary intake in a population

Traditional dietary assessment methods

One of the criticisms of population-based studies in nutritional epidemiology is the inability to accurately capture the foods consumed in the diet and their levels of intake, contributing to inconsistent evidence between (fermented) foods and health, and weakening their potential translation to clinical and public health applications (97, 98). Common dietary assessment methods include weighted food records (a detailed record of ingredients, foods, leftovers, and their weights), 24-h recalls (a detailed account of all foods consumed in the previous 24 hours self-reported by participants to trained interviewers), and food frequency questionnaires (FFQ) (self-reported intakes of a pre-determined list of foods, typically in the past month to year), each with their own advantages and drawbacks (99). In controlled intervention studies, multiple-day weighted food records are considered the 'gold standard' method for precisely measuring food intake during the study period. However, they may introduce reactivity bias (intentional or unintentional changes in dietary habits and health due to the reporting process), and their high respondent burden make them impractical to administer for a large population (100, 101). Another common method, the 24-h recall, is also regarded as being too time- and labor-intensive to administer to large populations, compounded by the fact that multiple recalls are often required to capture the day-to-day variation of foods and nutrients to reflect habitual intake (102). FFQs are typically the preferred method for measuring habitual intake in large population studies, since they can be administered and processed fairly efficiently for several thousand participants at once. However, the limited number of foods that can be assessed (and in detail), inaccurate estimation of portion sizes, and errors in food composition tables also make FFQ prone to measurement error (103, 104). Additionally, since both FFQs and 24-h recalls rely on (subjective) self-reporting, their precision depends upon the devotion, diligence, and memory of the participants. Such errors can lead to reduced power, confounding due to systematic reporting errors, as well as underestimated or overestimated findings in association studies.

Food intake biomarkers as objective measures of intake

To circumvent the limitations of self-report dietary measurement tools, researchers have looked to nutritional biomarkers as objective measures of dietary intake (105). In nutritional research, several definitions of biomarkers have been proposed, including 'a biochemical indicator of dietary intake/nutritional status (recent or long term), or an index of nutrient metabolism, or a marker of the biological consequences of dietary intake' (106, 107), and 'test results related to exposure, susceptibility or biological effect' (108). Beyond definitions based on technology or outcomes, biomarkers can also be classified based on their intended use. For instance, food intake biomarkers (FIBs) can be used to assess the intake of a specific food with a distinct dose- and time-dependent response, while effect biomarkers can help indicate a physiological or health state following the exposure to a food or diet (109). FIBs can also be used to monitor compliance to interventions in clinical trials (110), and may provide valuable information beyond self-reported intake particularly when food composition data are not available or limited.

Identifying a single FIB for a specific food can be a challenging task, since most dietary compounds commonly occur in many different foods. Different food sources may also contain common FIBs from a shared food processing method or metabolic pathway. This is particularly relevant to fermented foods, where common microbial fermentation pathways (*e.g.*, lactic fermentations) can result in the production of similar sets of metabolites. At the same time, the diverse raw material substrates used for fermentation can be a source of unique parent compounds. In these cases, multi-marker approaches, consisting of a combination of non-specific yet complementary biomarkers, could better inform the intake of fermented foods (111). Multi-metabolite panels have been suggested for wine, cocoa, and bread (111), but remain to be exploited for other fermented foods.

Further, the validation of FIBs is critical to ensure that they accurately represent a particular food, the level of intake of the food, and the intended use (112). Validation of a FIB involves several steps, often addressed across multiple studies. Naturally, the FIB should be food-derived and food-specific (plausibility), the biomarker should be able to distinguish between a range of intakes, and the kinetics of the FIB should be known and concordant with different levels of intake (dose-response) as well as its intended use (time-response). The FIB should also perform well in a free-living population after the intake of complex meals reflecting the habitual diet of the study population (robustness), and meet proper sampling, storage, and accuracy, sensitivity and specificity for the analytical equipment (validation criteria of reliability, stability, analytical performance, and reproducibility) (112). To date, only a small handful of biomarkers have been described as validated. These include the 'recovery biomarkers' doubly-labelled water for energy expenditure (113), 24-h urinary nitrogen for total protein intake (114), and 24-h urinary potassium for potassium intake (115), and 'concentration biomarkers' such as carotenoids for the intake of vegetables and fruits (116, 117), and n-3 fatty acids for the intake of oily fish (118). No validated biomarkers exist for the intake of various fermented foods.

The value of metabolomics in the discovery of food intake biomarkers

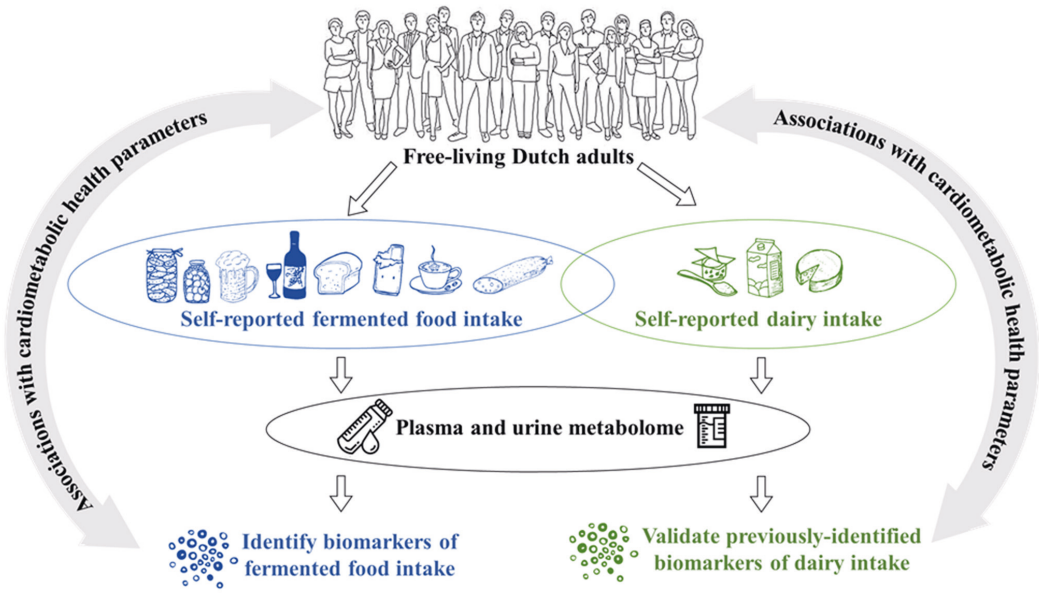
The identification of new FIBs has been driven by the application of metabolomics in nutritional research, which allows for a comprehensive measurement of all low molecular weight molecules in biological samples (119). The predominant analytical platforms used to detect and measure biomarkers can be described as either nuclear magnetic resonance (NMR)-based or mass spectrometry (MS)-based (*i.e.*, liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS)). A combination of analytical platforms is particularly valuable for biomarker discovery, and is widely used in nutritional metabolomics (120). The primary advantage of using combined platforms is that unique sets of FIBs can be identified based on the strengths of a particular platform, while simultaneously enhancing the confidence and accuracy of metabolites identified across multiple platforms. At the same time, certain disadvantages arising from the use of a sole platform can be mitigated (*i.e.*, GC-MS, high reproducibility of mass spectra but not suitable for non-volatile and heat-labile metabolites; LC-MS, high sensitivity and wide dynamic range but difficult to identify unknowns; NMR, good structural elucidation for identification but low sensitivity) (121). Guidelines on the best practices for metabolomics analyses in nutritional research – outlining study design, pre-processing, sample preparation, measurement, data analysis, compound identification, and biological interpretation – has been provided by members of the FoodBAll Consortium (120).

Objectives and outline of this thesis

Evidently, there is value in identifying FIBs for fermented foods that could improve the dietary assessment of these foods for future studies, inform on their nutritional quality, and elucidate the mechanisms of action that underpin the health benefits of fermented foods. In addition, there is a need to validate several candidate FIBs for fermented foods have emerged from non-targeted and targeted nutritional metabolomics studies, in real-life, non-controlled, situations. In particular, this includes several FIBs for fermented and non-fermented dairy products that were identified in short-term human dietary intervention studies conducted at Agroscope (34, 122-124). The overarching objective of this thesis (the *Cardioferment* project) is thus to further identify and validate candidate FIBs for fermented food intake using a real-life prospective cohort study in the Netherlands, "Nutritional Questionnaire plus" (NQplus), and examine their associations with cardiometabolic health.

The NQplus study is a prospective cohort study comprising 2048 primarily Caucasian Dutch adults (20 to 70 years), living in or around Wageningen, The Netherlands. It was initiated as an 'add-on' study to the National Dietary Assessment Reference Database (NDARD) project, to gather extensive data on participant demographics, diet (FFQ and 24-h recalls), lifestyle, medical history, and cardiometabolic health outcomes (125, 126). Fasting blood samples and 24-h urine samples were also collected. The study participants were recruited and included in the study between June 2011 and February 2013. All measurements were conducted at baseline repeated at 1 and 2 years of follow-up.

Figure 3. Schematic overview of the objectives of this thesis.



A schematic overview of the objectives of this thesis is provided in **Figure 3**. These objectives will be investigated across several chapters. In **Chapter 2,** a systematic review of the literature was performed to look for existing biomarkers of globally-consumed fermented foods. In **Chapter 3**, the prevalence of fermented food intake in the Netherlands was examined from systematically evaluating food lists from the NQplus FFQ and 24-h recalls, and assessed the relative validity of the FFQ in estimating the intake of fermented foods. In

Chapter 4, previously-identified FIBs for milk, cheese, and yoghurt were targeted in the plasma and urine metabolomes of a subcohort of NQplus participants, and their robustness was evaluated in a free-living population. In **Chapter 5**, associations were examined between dairy intake, a targeted panel of milk-derived free fatty acids, and CMD risk parameters. In **Chapter 6,** non-targeted metabolomics was applied to identify FIBs of fermented foods in the plasma and urine of a selected number of NQplus participants, and their associations with CMD risk parameters was examined. Finally, in **Chapter 7**, the main findings across all studies will be synthesized and discussed.

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List of abbreviations

AAB, acetic acid bacteria; ACE, angiotensin-converting enzyme; CFU, colony forming units; CHD, coronary heart disease; CLA, conjugated linoleic acid, CMD, cardiometabolic disease; CVD, cardiovascular disease; EU, European Union; FFQ, food frequency questionnaire; FIB, food intake biomarker; GC-MS, gas chromatography mass spectrometry; HDL, high-density lipoprotein; HR, hazard ratio; ISAPP, International Scientific Association for Probiotics and Prebiotics; LAB, lactic acid bacteria; LC-MS, liquid chromatography mass spectrometry; LDL, low-density lipoprotein; NDARD, National Dietary Assessment Reference Database; NMR, nuclear magnetic resonance; NQplus, Nutritional Questionnaire plus.

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CHAPTER 2

A systematic review to identify biomarkers of intake for fermented food products

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Abstract

Background: Fermented foods are ubiquitous in human diets and often lauded for their sensory, nutritious, and health-promoting qualities. However, precise associations between the intake of fermented foods and health have not been well-established. This is in part due to the limitations of current dietary assessment tools that rely on subjective reporting, making them prone to memory-related errors and reporting bias. The identification of food intake biomarkers (FIBs) bypasses this challenge by providing an objective measure of intake. Despite numerous studies reporting on FIBs for various types of fermented foods and drinks, unique biomarkers associated with the fermentation process ('fermentation-dependent' biomarkers) have not been well documented. We therefore conducted a comprehensive, systematic review of the literature to identify biomarkers of fermented foods commonly consumed in diets across the world.

Results: After title, abstract, and full-text screening, extraction of data from 301 articles resulted in an extensive list of compounds that were detected in human biofluids following the consumption of various fermented foods, with the majority of articles focussing on coffee (69), wine (69 articles), cocoa (62), beer (34), and bread (29). The identified compounds from all included papers were consolidated and sorted into FIBs proposed for a specific food, for a food group, or for the fermentation process. Alongside food-specific markers (*e.g.*, trigonelline for coffee), and food-group markers (*e.g.*, pentadecanoic acid for dairy intake), several fermentation-dependent markers were revealed. These comprised compounds related to the fermentation process of a particular food, such as mannitol (wine), 2-ethylmalate (beer), methionine (sourdough bread, cheese), theabrownins (tea), and gallic acid (tea, wine), while others were indicative of more general fermentation processes (*e.g.*, ethanol from alcoholic fermentation, 3-phenyllactic acid from lactic-fermentation).

Conclusions: Fermented foods comprise a heterogeneous group of foods. While many of the candidate FIBs identified were found to be non-specific, greater specificity may be observed when considering a combination of compounds identified for individual fermented foods, food groups, and from fermentation processes. Future studies that focus on how fermentation impacts the composition and nutritional quality of food substrates could help to identify novel biomarkers of fermented food intake.

Background

Fermentation as a food processing technology has been used for millennia to enhance the flavour, texture, and nutritive value of foods, as well as to improve their transportability, storage time, and/or safety (1, 2). Fermentation techniques continue to be refined and applied to a wide range of foods, including milk, grains, legumes, fruits, vegetables, fish, and meat products. Common types of fermented foods vary by region; for example, fermented dairy products (*e.g.*, cheese, yoghurt, buttermilk) are produced and consumed abundantly in Europe, fermented pulses and cereals (*e.g.*, dosai, idli, injera) are mostly indigenous to South Asia and Africa, and fermented soy products (*e.g.*, natto, miso, soy sauce, doubanjiang) are particularly common in East Asia (3-5). Other products are less regionally- or culturally-dependent, such as fermented fish products that are consumed in Korea (sikhae) and Japan (narezushi), as well as Sweden (surströmming) and Norway (rakfisk) (4, 6). The endless combination of foods (or 'substrates'), microorganisms, and fermentation techniques results in global fermented products with vastly different sensory and nutritional profiles. Currently, over 5000 types of fermented foods and beverages exist worldwide (7), and continued growth of the fermented food market is anticipated and fuelled by health food trends and rejuvenated artisanal practices.

Fermented foods are produced by the controlled growth and enzymatic activities of microorganisms, through four main fermentation processes (lactic, acetic, alcoholic, and alkaline) (4, 8). Lactic fermentations are carried out by lactic acid bacteria (LAB) (predominantly *Lactobacillus*, *Streptococcus*, *Pediococcus*, and *Leuconostoc*) for the production of fermented dairy, meat, and vegetable products, whereas acetic acid bacteria (*e.g.*, *Gluconacetobacter*) are responsible for the fermentation of cocoa, vinegar, and kombucha (9, 10). Alcoholic fermentations are driven by yeasts (*e.g.*, *Saccharomyces cerevisiae)* for the production of beer, wine, and breads, while alkaline fermentations make use of fungi (*e.g.*, *Penicillium spp* and *Aspergillus spp*) during the production and maturation of cheese, fermented meats, and fermented soy products (11, 12). Irrespective of the type of fermentation, microbial enzymes interact with the food matrix to produce novel metabolites, which can affect the sensory and functional profile of foods (13-20), and have also been suggested to possess bioactive qualities that can help prevent chronic diseases such as type II diabetes and cardiovascular disease (17).

Fundamental research suggests that the protective effects of fermented foods may be explained by fermentation-induced increases in the bioavailability of certain macro- and micronutrients (*e.g.*, protein, vitamins) (21), fermentation-induced decreases in anti-nutritional compounds (22), or driven by novel bioactive compounds of microbial metabolism (7). While several human observational studies have indicated a possible beneficial association between fermented food consumption and cardiometabolic health, specifically in terms of weight maintenance, diabetes/glucose homeostasis, and overall cardiovascular disease risk (23-25), the evidence is still inconclusive, in part due to the limitations of tools used to quantify fermented food intake.

Currently, self-report food frequency questionnaires (FFQs), 24-h recalls, and food diaries (weighed or unweighed) are the most commonly used dietary assessment tools to quantify food intake. The FFQ is usually the method of choice in observational cohort studies. In contrast to diaries and recalls, FFQs are relatively easy to administer and process, but their accuracy relies on the memory and devotion of respondents. Consequently, random and systematic errors, including memory-related bias, incorrect estimates of portion sizes, and/or bias towards socially desirable answers, are inevitable (26, 27). There is also no FFQ that has been specifically designed to estimate fermented food intake, and food lists in existing FFQs may not comprehensively cover the intakes of this diverse food group, or distinguish the nuances within specific foods that affect their fermentation status (*e.g.*, fermented pickles vs. acidified pickles). Moreover, none of the self-report dietary assessment methods take into account differences in food metabolism between individuals, which can have a significant bearing on the immediate effects of diet and subsequent health consequences. The importance of accurately assessing food intake across diverse populations has propelled food intake

biomarkers (FIBs), as promising 'objective' measures of intake and metabolism, to the forefront of dietary assessment research (26, 28).

While the identification of a single specific biomarker is ideal, this is not always possible due to the overlapping characteristics shared by many foods. A number of combination biomarkers have thus been proposed, for example in the case of red wine (tartaric acid reflecting the grape raw material, plus ethyl glucuronide reflecting alcoholic fermentation and phase II metabolism) (29). A similar approach is expected to be suitable for identifying FIBs for other fermented food products, such that a group of compounds, although not unique to the food themselves, might be useful in combination to stratify between high and low consumers of different fermented foods in intervention studies and epidemiological cohorts.

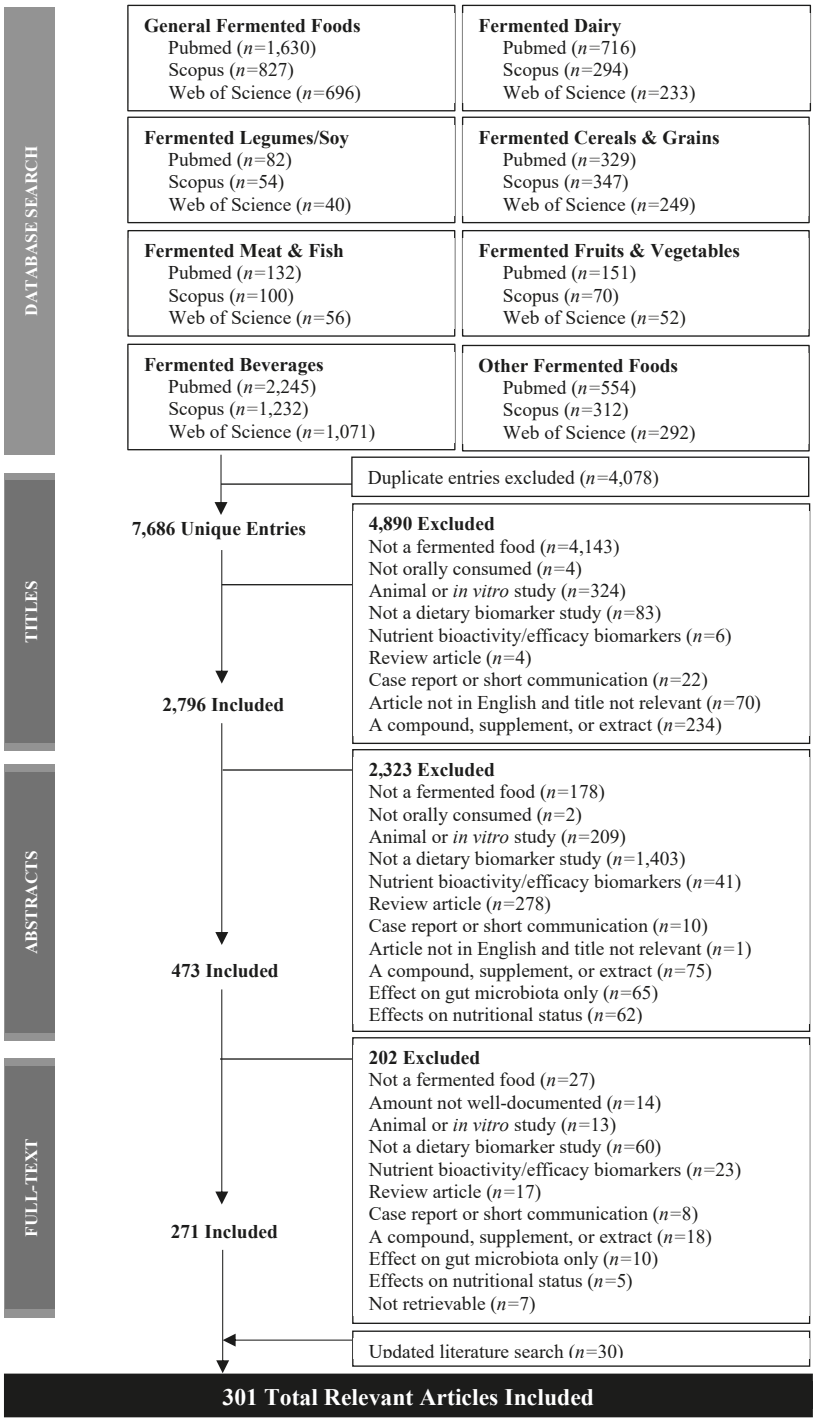
The Food Biomarker Alliance (FoodBAll) (28), a project funded by the Joint Programming Initiative a Healthy Diet for a Healthy Life, has set guidelines for identifying and validating FIBs (30) (http://foodmetabolome.org) (31). This effort has resulted in a systematic documentation of FIBs for major food groups, including fruit and vegetables, meats, fish, and other seafood, dairy products, cereals and whole grains, alcoholic and non-alcoholic beverages, vegetable oils, nuts, and spices and herbs (32-40). The purpose of the current systematic review is to present a comprehensive overview of compounds reported in the literature that could, alone or in combination, represent FIBs for various fermented foods. We anticipated that identified compounds could be stratified into FIBs at the food level, food group level, and fermentation level, to discriminate a dietary pattern of fermented food consumption.

Methods

Primary database search

The literature search strategy and search terms were developed in accordance with the guidelines previously proposed by the FoodBAll consortium (30), and all elements of the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement relevant for a literature search on biomarkers were reported (41). Primary articles were identified from PubMed, Scopus, and ISI Web of Knowledge. In order to obtain a broad coverage of fermented foods products consumed globally, eight food groups were defined for the search strategy, specifically: (i) general fermented foods, (ii) fermented dairy, (iii) fermented meats and fish, (iv) fermented fruits and vegetables, (v) fermented legumes (including soy), (vi) fermented cereals and grains, (vii) fermented beverages, and (viii) other fermented products (*e.g.*, chocolate, condiments and sauces). These food groups were loosely based on the food-based dietary guidelines in The Netherlands, Switzerland, and United States (42-44), but were inclusive of fermented food items consumed worldwide. Individual fermented foods were further specified within each food group, as detailed in **Table S1**. Exclusion terms were individually applied to each search to limit the number of false-positive hits. Each of the eight food group terms was searched for in conjunction with a combination of search operators, as detailed in **Table S2**. The search fields applied were [Title/Abstract] for PudMed, [Title/Abstract/Keywords] for Scopus, and [Topic] for ISI Web of Knowledge. All searches were conducted in October 2018, and an updated literature search was performed in September 2020. No restrictions were applied on the publication date. Furthermore, the reference lists of relevant systematic reviews and meta-analyses (32-34) were scanned for relevant articles for inclusion. The full literature search process is outlined in **Figure 1**.

Figure 1. Schematic Outline of the Systematic Literature Search.



Inclusion and exclusion criteria

Search inclusion and exclusion criteria were defined *a priori.* Studies were included if the primary exposure was oral consumption of a fermented food, where 'fermented food' was defined according to the definition given by Marco *et al*.: 'foods or beverages made through controlled microbial growth and enzymatic conversions of major and minor food components' (8). The study had to be conducted in humans, and report on compounds that could be detected in biosamples following consumption of a fermented food. Studies were excluded if: the food being considered was not fermented or if it was unclear if the food was fermented; the route of exposure to the food was not oral consumption; the amount of food consumed was not well-documented (*e.g.*, a gram amount, or categorization to distinguish the fermented food from other foods consumed was not provided); the study was conducted in animals or *in vitro*; compounds in biological samples that could represent food biomarkers were not described; the aim of the study was to either review nutrient bioactivity and nutritional status using the fermented food as a delivery matrix, or to assess the impact of a fermented food on bioavailability of another food/compound; the study focused on a compound, supplement, or extract rather than a whole food; or the study only investigated alterations on the composition of the gut or fecal microbiota. Review articles, case reports or short communications (*e.g.*, comment, editorial, conference abstract), and articles in a language other than English were also excluded.

Strategy to identify and select the most discriminant compounds

Since our goal in this review was to evaluate a combination of candidate biomarkers for fermented foods (both specific and non-specific), rather than a single specific biomarker, we slightly deviated from the assessment of validity for putative/candidate biomarkers that was previously proposed by Dragsted *et al.* (31). Following the selection of relevant full-text articles for inclusion, a series of steps were applied to select the most discriminant candidate biomarkers from the literature search. These included compounds that were highly discriminant for (i) the food ('food-level' biomarkers – *i.e.*, FIBs specific for the intake of a particular food), (ii) food group ('food group-level' biomarkers – *i.e.*, FIBs specific for the intake of a group of foods with a common raw material substrate or characteristic), or (iii) a dietary pattern of fermented food consumption ('fermentation-dependent' biomarkers – *i.e.*, FIBs arising from the fermentation process of a food), from other non-fermented foods and food groups. In order to capture both specific markers that can discriminate the intake of a fermented food as well as non-specific markers that may act in combination to discriminate the intake of a fermented food, we firstly focused on summarizing in detail the compounds that were identified in discovery-driven 'untargeted' studies that typically employed metabolomics tools (58 articles), and supplemented this information with 'targeted' studies investigating a particular compound or set of compounds (243 articles), analysed using metabolomics tools or other biochemical assays. Information from the untargeted studies was expected to identify biomarkers associated with a dietary pattern of consuming fermented foods, while information from both the untargeted and targeted studies was expected to help to further identify and verify biomarkers at the food level (*e.g.*, cheese) and food group level (*e.g.*, fermented dairy).

Compounds were selected if they were statistically significantly increased following consumption of the fermented food compared to baseline or control, and/or have been detected in multiple studies. For these selected compounds, we further consulted the study text to assess the biological plausibility, along with previous FIB reviews for their validation status. In addition, three food/metabolite databases (HMDB, Exposome-Explorer, FooDB) were searched in May 2019 and updated in September 2020 as an additional step for verifying that a compound appearing in a biosample has a food origin (or was transformed during metabolism) and to check the specificity of the compound for a fermented food. Information from food databases and the wider literature were also used to identify and confirm metabolites of fermentation. Compounds that were not discriminative of these classifiers but were associated with the fermented food or food group (*e.g.*, detected but not significantly increased in biosamples following consumption), or compounds

which have a ubiquitous presence across many other non-fermented foods, were not selected and not further discussed.

Results and Discussion

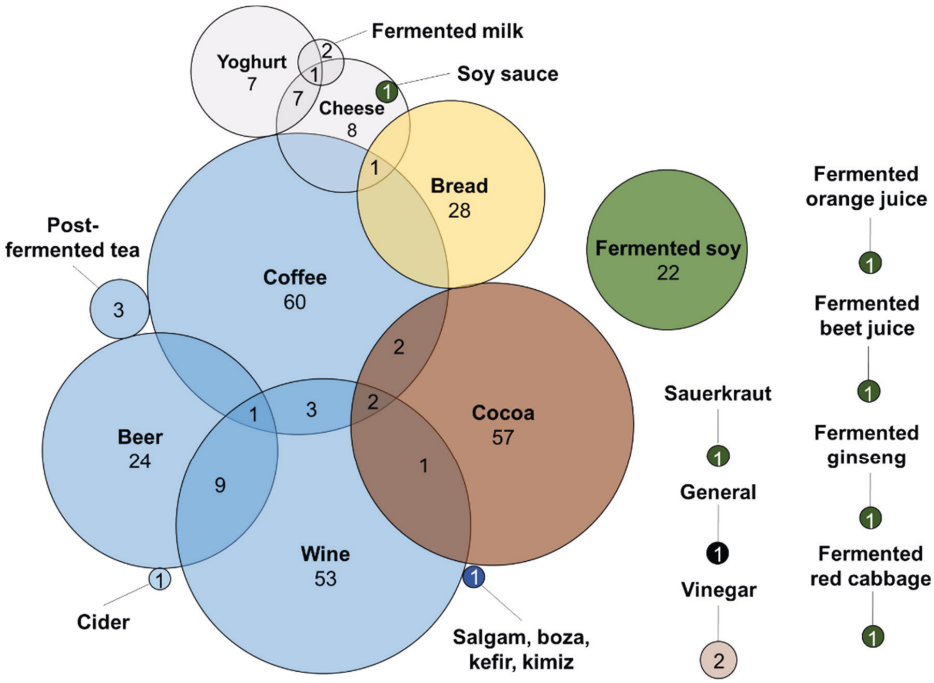
Database search

From the initial primary database search, a total of 11,764 records were identified, of which 7,686 unique entries remained following the removal of duplicates (**Figure 1**). After filtering the 7,686 titles, 4,890 were excluded and 2,794 were deemed relevant for further review and their abstracts were retrieved. Following abstract review, 473 relevant entries remained, and their full-text articles were retrieved (2,323 were excluded for various reasons outlined in the exclusion criteria). Further application of exclusion criteria to full-text articles (202 articles removed), and an updated search (30 articles added), resolved in 301 relevant full-text articles with information on compounds associated with intake of various fermented foods. The fermented foods investigated in the $n = 301$ studies were: coffee ($n = 69$), wine ($n = 69$), cocoa ($n = 62$), beer ($n = 34$), bread ($n = 29$), fermented soy ($n = 22$), cheese ($n = 18$), yoghurt ($n = 15$), fermented milk ($n = 3$), post-fermented tea ($n = 3$), vinegar ($n = 2$), cider ($n = 1$), traditional Turkish beverages (salgam, boza, kefir, and kimiz) ($n = 1$), fermented orange juice ($n = 1$), fermented ginseng ($n = 1$), fermented beet juice ($n = 1$), fermented red cabbage ($n = 1$), soy sauce ($n = 1$), sauerkraut ($n = 1$), and general fermented products ($n = 1$) (**Figure 2a**). The numbers of identified metabolites reported for each food across these studies are presented in **Figure 2b**, and detailed lists all of the included articles are presented in **Table S3** (untargeted studies) and **Table S4** (targeted studies). No studies reported on potential FIBs for fermented meat or fish products. Biological samples in which putative FIBs were identified or measured included serum (14 untargeted, 28 targeted studies), plasma (15 untargeted, 120 targeted studies), whole blood (13 targeted studies), urine (33 untargeted, 125 targeted studies), feces (5 untargeted, 7 targeted studies), ileal fluid (5 targeted studies), subcutaneous adipose tissue (2 targeted studies), oral fluid (3 targeted studies), plasma lipoproteins (2 targeted studies), erythrocytes (2 untargeted studies), capillary blood (1 targeted study), breast milk (1 targeted study), hair (1 targeted study), and breath (1 targeted study). The majority of studies were postprandial intervention studies ($n = 183$). The remainder comprised short-term and long-term intervention studies ($n = 83$) and observational studies where participants followed their habitual diet (where the diet was assessed by self-report tools such as FFQ, recall, food record, or dietary history) ($n = 53$).

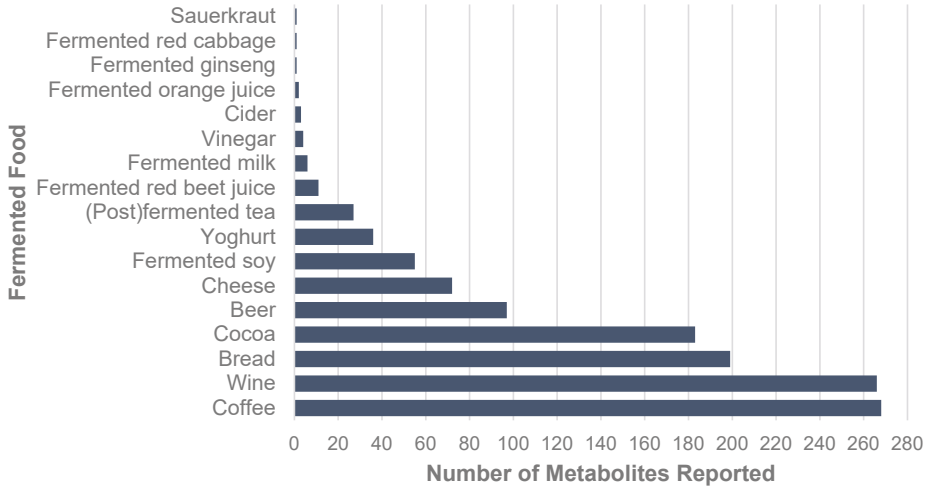
From these relevant publications, compounds that could represent FIBs for various fermented foods are discussed based on their classification into three categories: food-level, food group-level, and fermentation-dependent FIBs (as described in the methods). An overview of the main FIBs identified, selected, and classified in this search is provided in **Table 1**. Although the aim of this review was not to identify a complete list of food-level or food group-level biomarkers, their inclusion in this review alongside fermentation-dependent markers provides the basis to help to facilitate a so-called multi-marker approach in estimating fermented food intake. Such a multi-marker approach could help confirm fermented food intake, or help distinguish between the intake of fermented and non-fermented versions of the same food.

Figure 2. Overview of 301 included publications from the systematic literature search. (a) Number of publications identified for each type of fermented food (colored by food group). No articles were identified for fermented meat or fish products. (b) Number of identified metabolites reported in the included articles for the fermented food described.

(a)



(b)



Food-level biomarkers

Due to the overlapping compositional profiles of many foods, identification of specific FIBs for individual foods is challenging. In this review, food-level biomarkers were identified for beer, bread, wine, coffee, cheese, and fermented (rooibos) tea (**Table 1**). These compounds were largely derived from the (unfermented) raw materials. For example, isoxanthohumol, 8-prenylnaringenin, and *iso*-alpha-acids originate from beer hops that are used in the brewing process, tartaric acid and resveratrol are found at high concentrations in the grapes used for wine production (45-48), trigonelline and 2-furoylglycine originate from coffee beans and the coffee roasting process (49, 50). For bread, the organic acids 2,4-dihydroxybutanoic acid and 2,8-dihydroxyquinoline glucuronide were identified following the intake of fermented sourdough endosperm rye and white wheat bread (52). While these organic acids have seemingly not yet been detected/quantified in other foods (from food database searches), future validation would useful in determining their usefulness as specific biomarkers for bread intake.

Food-specific biomarkers for fermented dairy products (cheese, yoghurt, buttermilk) (34), coffee (33), and cocoa products (32, 53) have also been the subject of previous systematic reviews. Notably, isovalerylglutamic acid, isovalerylglycine, triglylglycine, and isobutyrylglycine were previously identified as specific FIBs for cheese (34). A large number of phenolic acid, alkaloid, and terpene derivatives have been identified as FIBs of coffee, with trigonelline and cyclo(isoleucylprolyl) emerging as the most specific biomarkers (33). No specific biomarkers were identified, previously or in the current review, for yoghurt, buttermilk, or cocoa; however, several non-specific biomarkers at the food group level were found.

Food group-level biomarkers

A number of FIBs previously proposed as food-specific markers have been re-classified as discriminant for a group of foods in light of evolving research. For instance, while caffeine has been consistently linked to coffee intake, it is also detected at fairly high concentrations in tea and chocolate (33, 53). In addition, a growing range of products can be artificially-caffeinated, which also obscures the use of caffeine as a FIB for only naturally-caffeinated foods. Similarly, the hydroxycinnamate ferulic acid has been detected in high levels in coffee (54), but it is also an antioxidant that is ubiquitously found in plant tissues (55). Unsurprisingly, increased levels of ferulic acid and its derivatives have been detected in blood and urine following the consumption of multiple plant-based foods (56, 57), indicating that the sole use of this compound as a FIB for coffee would be inappropriate. These compounds however may still be useful as food group-level biomarkers in conjunction with food-level and/or fermentation-dependent biomarkers for evaluating the intake of fermented foods.

The biomarkers identified at the food group-level for different fermented foods is summarised in **Table 1** and their relevance discussed below. It is important to note that while we defined ‘food groups’ in the literature search by those conventionally used in dietary recommendation guidelines, food group-level biomarkers may be common across multiple foods based on common substrates of fermentation (*e.g.*, wheat in both beer and bread production), or multiple biomarkers may apply in the case of multiple substrates for a single fermented food (*e.g.*, tarhana, a fermented mixture of cereals and yoghurt). Since fermented beverages encompass a broad, heterogenous group, different fermented beverages are discussed in the context of their fermentation raw material, which includes milk (fermented milks, kefir, and yoghurt-based drinks), fruits (cider, wine, fermented orange juice, fermented beet juice), cereals and grains (beer), and others (coffee, post-fermented tea). In many cases, compounds identified at the food group-level represented FIBs of unfermented raw material rather than a fermented food group, but nonetheless, their description is important as part of a combined model of fermented food intake.

a) Fermented dairy

Several compounds identified in our search were associated with the intake of cheese and yoghurt, including the widely discussed fatty acids heptadecanoic acid (C17) and pentadecanoic acid (C15). These fatty acids were also captured in the systematic review of egg and dairy biomarkers by Munger *et al.* (34) as dairy biomarkers, where additional FIBs were proposed for general dairy fat/dairy products, including 10Z-heptadecenoic acid (C17:1), myristoyl-sphingomyelin SM(d18:1/14:0), and galactonate. A handful of additional compounds that could represent biomarkers for milk (as compared to fermented milk) were additionally identified in the current search, including galactitol, galactonate, galactono-1,5-lactone, galactose, and lactose (58-60). Collectively, these compounds represent FIBs that may be useful for estimating total dairy intake, including both fermented and non-fermented dairy. As the degree of transformation of lactose (and similar metabolites) greatly varies among dairy products, the profile of these combined metabolites could provide specific insights into the degree of fermentation of the ingested dairy products.

It has been reported that fermentation of milk products may increase the bioavailability of nutritionally-important and bioactive compounds of milk (61). Major milk proteins include caseins (αs1, αs2, β and k), β-lactoglobulin, α-lactalbumin (precursor of serotonin), immunoglobulins (IgA, IgG, IgM), glycomacropeptide, lactoferrin, lactoperoxidase, lysozyme, and serum albumin (7). Milk proteins are easily hydrolysed to free amino acids during fermentation, and a large group of amino acids (alpha-amino butyric acid, alanine, asparagine, cysteine, glycine, glutamine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) were found to be increased in plasma following yoghurt and cheese intake compared to control (milk or water) (62-64). Dairy proteins are also a source of bioactive peptides that can be released during fermentation or during digestion (65, 66). The bioactive peptides derived from these milk proteins during fermentation, such as Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP), are reported to possess antimicrobial, antioxidative, immunomodulatory, angiotensin-1-converting enzyme (ACE-1) inhibitory and renin inhibitory activities (7). While these peptides were not identified in our search, their presence in fermented dairy products warrants further investigation in a combination biomarker approach for this food group.

b) Fermented cereals and grains

Cereals and grains are a staple agricultural product around the world, and their fermentation results in an array of rice-based (idli, dosa), wheat-based (bread, kishk), corn-based (ogi, pozol), or sorghum-based (injera, kisra) dishes (67). To date, the vast majority of research activity on FIBs of this fermented food group has centred around wheat-based bread products (whole or refined grain) that are leavened with baker's yeast (*Saccharomyces cerevisiae*), with little to no reports on FIBs for other fermented grains. In the current review, alkylresorcinols and their primary metabolites 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-propanoic acid (DHPPA), as well as benzoxazinoids and their metabolites (2-hydroxyl-1,4-benzoxazin-3-one, hydroxylated phenylacetamides and derivatives thereof) were identified as FIBs of wholegrain wheat/rye (68-73). Since these compounds are derived from wholegrain wheat and rye, they are present at a higher abundance in biosamples following consumption of wholegrain breads, rather than refined-wheat bread (68). A recent review focusing on mass-spectrometry analysis of whole grains revealed the presence of hundreds of molecules in various wheat, barley, oat, and rye products, including alk(en)ylresorcinols, benzoxazinoids, avenanthramides, flavonoids, lignans, phytosterols, carotenoids, phenolic acids (hydroxybenzoic and hydroxycinnamic acids), sphingolipids, tocols, and glycine betaine (74). While these compounds have been primarily reported in raw grains and leavened bread products, they may also be useful as FIBs for fermented food products in which grains are used as a starting raw material (*e.g.*, wheat/barley in beer production). It has not yet been investigated whether these compounds can also be detected in soy sauce, which is a fermented mixture of soybeans and wheat (67).

A further distinction should also be made for breads that are leavened solely by yeast, and sourdough breads, which are both leavened by yeast and fermented by LAB. Sourdough-fermented rye has also been shown to contain higher levels of organic acids compared to rye bread, which can reduce starch digestibility and gastric emptying rate, leading to reduced insulin and glucose responses (75, 76). In one study, consumption of sourdough fermented bread increased total free amino acids in plasma compared to bread fermented solely with yeast, indicating improved digestibility of protein (77).

c) Fermented meats and fish

Fermented meat products are broadly produced and consumed in Germany, France, Spain, Italy, the Balkans, Hungary, Australia, USA, and Japan (7). Despite their widespread consumption, no studies were identified in the current search that reported on candidate FIBs of fermented meat or fish. However, a number of studies have identified FIB of raw or unfermented meat and fish products. For example, a study in free-living individuals previously identified candidate biomarkers for chicken (anserine), meat (chicken, red meat, processed meat) (carnosine), fish (trimethylamine-N-oxide), and meat and fish intake (3-acetylcarnitines, including acetylcarnitine, propionylcarnitine, and 2-methylbutyrylcarnitine) (78). In another study, 1- and 3-methylhistidine were determined to be urinary biomarkers for meat intake (79). Furthermore, raw meat is known to contain the histidyl dipeptides, carnosine and anserine (80). FIBs for meat intake were comprehensively evaluated in a review, in which urea, creatine, creatinine, carnitine, carnosine, anserine, ophidine, 1- and 3-methylhistidine, and sulfate or sulphite were described as the most discriminant compounds (81).

For fermented meats, nitrites that are used as curing agents might also be present in some final products (82). In addition, some fermented sausages have been reported to contain high levels of the biogenic amine tyramine (83), and the antioxidant taurine (2-aminoethane sulfonic acid) (80, 84), both of which warrant confirmation as FIBs for fermented meat in human studies. Similarly to fermentation of other high-protein foods, fermentation of meat products also releases bioactive peptides from proteolytic protein degradation. ACE-1 inhibitory peptides and antioxidant peptides have been identified in cured ham and fermented sausages, such as Serbian Petrovac sausage (85) and Spanish dry-cured ham (86-88). While detected in the foods themselves, no studies were identified in the literature search in which these peptides were identified in biosamples following consumption of fermented meat products. On the other hand, biogenic amines (89) as well as ACE-1 inhibitory peptides (90) are well described in cheese, indicating that the distribution of these molecules extends beyond fermented meat products.

d) Fermented fruits and vegetables

While, in theory all fruits and vegetables could be fermented, those most commonly fermented include cabbage (sauerkraut, kimchi), cucumbers, olives, onions, carrots, caper berries, and garlic (91, 92). Fruits and vegetables are commonly fermented using LAB and yeasts via techniques such as dry salting or storage in a brine (91). During the lactic fermentation of cucumbers, cabbage, and olives, glucose and fructose are broken down to produce lactic acid, acetic acid, ethanol, and carbon dioxide (92). The production of organic acids plays a critical role in food safety by limiting the growth of pathogenic microorganisms (91). Slight differences in the fermentation process can also alter the final metabolite composition and quantities between food products. For instance, fermentation of cabbage into sauerkraut degrades glucosinolates to isothiocyanates, indole-3-carbinol, goitrin, allyl cyanide, and nitriles. While the degradation products allyl isothiocyaniate, allyl cyanide, and goitrin were higher in the spontaneously fermented product consisting of salted raw cabbage, methyl isothiocyanate and indole-3-carbinol were higher following in sauerkraut fermented with a starter culture containing LAB (93).

Plasma β-cryptoxanthin and lutein have been previously proposed as robust biomarkers for general fruit and vegetable intake (94) and have been used to measure dietary compliance in multiple human

intervention studies. Untargeted metabolomics studies have further revealed a wide range of compounds associated with the intake of plant-based foods (95). However, from our systematic search, only five studies investigating fermented fruits and vegetables were identified. In one study, D-phenyllactic acid, a LAB metabolite, increased in the serum and urine of four volunteers following acute consumption of sauerkraut (96). In another study, 20-O-beta-D-glucopyranosyl-20(S)-protopanaxadiol, a novel ginseng saponin metabolite, was increased following intake of fermented ginseng. However, it was reported that the formation of this compound is likely attributable to the action of human intestinal bacteria (97). Further, it was reported that fermented red cabbage has lower bioavailability of anthocyanins compared to fresh red cabbage (98). Contrary results were reported in Hornero-Mendez *et al*. (99), where bioavailability of beta-cryptoxanthin and lutein (both attributed to oranges) were higher following consumption of fermented orange juice, and in Sawicki *et al*. (100), where increased levels of betalain and derivatives in plasma and urine following the consumption of fermented red beet juice were all attributed to red beetroot. However, despite the higher bioavailability afforded by the fermented products, high intake of unfermented forms of these foods would greatly obscure their use as FIBs in dietary assessment.

e) Fermented legumes and soy

Although the current search focused on identifying FIBs for all fermented legumes including soy products, only studies on fermented soy products were identified. Soybean products are commonly produced and consumed in East and Southeast Asia, and West Africa (4). Plasma and urinary isoflavones have long been used as markers of soy exposure (101, 102), and more recently, pinitol was identified as candidate biomarker of soy intake in an untargeted metabolomics study (60). Although most soy products are characterized by their isoflavone content (which are also present at moderate levels in other legumes), fermented soybeans are comparatively richer than non-fermented soybeans in the isoflavone genestein, as well as gamma-polyglutamic acid (PGA) which is produced by some strains of *Bacillus subtilis* during fermentation (4). In addition, the natural isoflavones present in soybeans and unfermented soy products are glucose-conjugated, and converted to the aglycone-isoflavones following hydrolysis during digestion prior to absorption (51). Aglycone-enriched isoflavones that are present in fermented soy products has been reported to be more efficiently absorbed and therefore more bioavailable (51). In a study by Jang *et al.* (103), comparing levels of soy isoflavones following ingestion of test meals containing fermented or unfermented soybean, the metabolites daidzein 7-O-glucuronide-4′-O-sulfate and genistein 4′,7-di-O-glucuronide were significantly higher in plasma, and genistein 7-Osulfate, glycitein 7-O-glucuronide-4′-O-sulfate, and genistein 4′-O-sulfate were significantly higher in urine, following fermented soy consumption, indicating these metabolites may be useful in distinguishing soy products with different fermentation status. In another acute intervention study, it has been demonstrated that fermentation of soybean increases the urinary recovery of soy isoflavones by 52% (104). Analysis of several fermented soy products, including Chungkookjang, tempeh, doenjang, and miso, revealed higher levels of isoflavones (genistin, daidzin, glycitin, genstein, daidzein) and/or amino acids (in particular glutamate) compared to unfermented soybean (105). In addition to soy isoflavones and aglycones, vitamins B2 and B12, and gamma aminobutyric acid (GABA), are increased in fermented soy products (106), and a variety of bioactive peptides have been identified, such as F2-2-2 and Fr-2-3 in chungkujang, Arginine-Proline in doenjang, Phenylalanine-Isoleucine-Glycine (1:2:5) in dou-chi, and Valine-Proline-Proline and Isoleucine-Proline-Proline in miso paste containing casein (107-109). While many of these compounds are present across other foods as well (*e.g.*, vitamin B12), which limits their usefulness as FIBs for fermented soy intake, their combination in a multi-marker approach warrants investigation.

f) Other fermented products

Coffee, tea, and chocolate are consumed worldwide, but largely unbeknownst to consumers as 'fermented' food products. Unlike yoghurt and cheeses, where the final food products are subject to fermentation and are typically carriers of live microorganisms, fermentation of coffee, tea, and cocoa occurs upstream in the food manufacturing process (110). Following their harvest, cocoa seeds are intentionally fermented for 7 days (111, 112), raw coffee berries for 10 to 25 days (113), and in the case of post-fermented teas, fresh tea leaves may be fermented from several months up to several years (114). These foods rely on spontaneous fermentation via the actions of endogenous microbes, and depending on the duration and conditions of the fermentation, different compositional and flavour profiles are attained.

Along with the food-level biomarkers identified for coffee, post-fermented tea, and cocoa as described above, our systematic search revealed several overlapping candidate biomarkers for these foods based on a common raw material characteristic other than a shared substrate. These included caffeine and its metabolites (theobromine, theophylline, methylxanthines, methylurates), nicotinic acid, and multiple phenolic acids, including (epi)catechin, chlorogenic acid, caffeic acids, and quinic acids (95, 115-118) (**Table 1**). Polyphenols are a group of chemically-diverse compounds with high abundance in the diet (115, 116). Despite the widespread prevalence of polyphenols in a variety of plant-based foods (*i.e.*, coffee, wine, citrus, apples, pears, tea, chocolate), which renders them non-specific biomarkers, distinct polyphenols have been shown to be more closely associated with certain foods than others. For example, methyl-(epi)catechin sulfate has been associated with chocolate intake, hydroxytyrosol, resveratrol, and gallic acid with red wine intake, and (dihydro)ferulic acid and caffeic acid with coffee intake (115, 116). Quantification of these polyphenols in biofluids may assist in determining cut-offs or ratios as an indication of their usefulness as biomarkers of acute or habitual intake of these foods. Furthermore, enterolactone, a phytoestrogenic compound formed via gut microbial transformation of plant lignans, has been detected in the blood or urine of individuals following consumption of breads, cocoa, coffee, and tea, and soy products. The non-specific nature of this compound limits its usefulness as a specific FIB, but may be interesting to explore as a food group-level biomarker.

Table 1. Candidate FIBs identified for various fermented foods from the systematic literature search

Fermented Food(s)	Discriminant Compounds/Candidate Biomarker Level[a]			
	Food-Level	**Food Group-Level[b]**		**Fermentation-Dependent**
Wine	• Tartaric acid/tartrate [**grapes**] • Resveratrol and metabolites (trans-piceid, glucuronides and sulfates) [**grapes**]	• (Epi)catechin and metabolites [**polyphenol**] (also see cocoa, coffee, tea)		• Ethanol [**alcoholic fermentation**] • Ethyl glucuronide [**ethanol metabolism**] • Ethyl sulfate [**ethanol metabolism**] • Mannitol [**wine fermentation**] • Gallic acid [**EGCG fermentation**]
Beer	• (Iso)xanthohumol [**hops**] • Iso-alpha-acids (isohumulones) [**hops**] • 8-Prenylnaringenin [**hops, metabolite of isoxanthohumol**]	• Alkylresorcinols and metabolites (3,5-dihydroxybenzoic acid, DHPPA, C17 to C21:0 ratio) [**wholegrain wheat/rye**] • Benzoxazinoids and related compounds (2-hydroxyl-1,4-benzoxazin-3-one, hydroxylated phenylacetamides and derivatives, HHPAA glucuronide and sulfate) [**wholegrain wheat/rye**]	• Ferulic acid, dihydroferulic acid, and derivatives [**plant phenol**]	• 2-Ethyl malate [**beer fermentation**]
Bread	• 2,4-Dihydroxybutanoic acid* [**organic acid**] • 2,8-Dihydroxyquinoline glucuronide* [**intermediate in gut microbial metabolism of quinoline or 8-hydroxyquinoline**]			• Methionine [**sourdough fermentation, LAB/yeast fermentation**]
Cocoa	• None identified	• Caffeine and metabolites (theophylline, 1-Methylxanthine, 3-methylxanthine, 7-methylxanthine, paraxanthine, theobromine, AAMU, AMMU) [**naturally or artificially-caffeinated products**] • 1-, 3-, or 7-Methyluric acid, 1,3-, 1,7-, or 3,7-dimethyluric acid, 1,3,7-trimethyluric acid [**caffeine/theophylline metabolism**] • Chlorogenic acid, caffeic acids, quinic acids [**hydroxycinnamates**] • Nicotinic acid, hydroxynicotinic acid [**vitamin B3 metabolism**] • (Epi)catechin, (epi)catechin glucuronide and metabolites (3-hydroxyhippurate, MHPV, MHPV sulfate, glucuronide, 4-hydroxy-5-(3,4-dihydroxyphenyl) valeric acid, 4-hydroxy-5-(hydroxyphenyl) valeric acid sulfate, DHPV glucuronide, sulfoglucuronide) [**polyphenol metabolism**]		
Coffee	• Trigonelline [**coffee beans**] • N-methylpyridinium **[trigonelline metabolite, from roasting]** • Cyclo(isoleucyl-prolyl) • Atractyligenin glucuronide • 2-Furoylglycine [**furan metabolites, roasting**] • 4-Ethylguaiacol • 4-Vinylguaiacol			• Acetate/acetic acid [**acetic acid fermentation**]
Tea[c]	• C-linked dihydrochalcone and flavanone glucosides [**specific to rooibos**]			• Theabrownins [**alkaline fermentation of tea polyphenols**] • Gallic acid [**EGCG fermentation**]

Soy[c]	• None identified	• Pinitol [**soybeans, legumes**] • Isoflavones (daidzein, genistein, glycitein), glycoside-enriched [**soybeans, legumes**]	• Aglycone-enriched isoflavones and certain 4' and 7' isoflavone metabolites [**fermentation**] • Threonine, Tryptophan, Tyrosine, Valine [**alkaline fermentation**] • Vitamin B12 [**alkaline fermentation**] • Indole-3-lactic acid [**LAB fermentation**]	• 4-Methylspinacemine [**fermentation**] • Menaquinone-7 (vitamin K2) [**fermentation**]
Cheese	• Isovalerylglutamic acid • Isovalerylglycine • Triglylglycine • Isobutyrylglycine	• Pentadecanoic acid (C15) [**milk fat**] • Heptadecanoic acid (C17) [**milk fat**] • 10Z-Heptadecenoic acid (C17:1) [**milk fat**] • Myristoyl-sphingomyelin SM(d18:1/14:0) [**milk fat**] • Lactose [**milk**] • Galactitol [**milk**] • Galactonate [**milk**] • Galactono-1,5-lactone [**milk**] • Galactose [**milk**]	• 3-Phenyllactic acid [**LAB fermentation**] • Methionine [**LAB/yeast fermentation**]	• Lactic acid [**LAB fermentation**]
Yoghurt	• None identified		• Indole-3-lactic acid, indole-3-acetaldehyde, indole-3-propionic acid [**LAB fermentation**]	

AAMU = 5-acetylamino-6-amino-3-methyluracil; AMMU = 5-acetylamino-6-formylamino-3-methyluracil; DHPV = 5-(3,4-dihydroxyphenyl)-γ-valerolactone; DHPPA = 3-(3,5-dihydroxyphenyl)-1-propanoic acid); HHPAA = 2-hydroxy-N-(2-hydroxyphenyl) acetamide; LAB = lactic acid bacteria; MHPV = 3′-methoxy-4′-hydroxyphenylvalerolactone.

[a] Wherever possible, the raw material from which the metabolite is derived from, the chemical class, or the fermentation or metabolic process by which the metabolite is generated from, is indicated in square brackets. A full list of references from which these metabolites were derived is provided in Tables S3 and S4. The specificity of food-level FIBs for each fermented food (or raw material) was verified through food database searches. Where specificity could not be confirmed, the metabolite is marked with a (*), and further expanded upon in the text.

[b] A group of foods with a common raw material substrate or characteristic.

[c] Post-fermented tea, and fermented soy products.

Fermentation-dependent biomarkers

Fermentation of foods is used in part to improve the bioavailability of dietary compounds, or release novel metabolites generated via microbial enzymes (8). These metabolites that can be considered as potential fermentation-dependent FIBs associated with a dietary pattern of fermented food consumption have not been previously documented in a systematic manner. In this review, we identified several compounds that arise from the fermentation process of a particular food, food group, or different fermented foods possibly indicating fermentation with common microbes.

Several of the potential FIBs identified in this search correspond to specific features of the type of fermentation process or the food that is fermented. Notably, the presence of high levels of the sugar-alcohol mannitol in wine is indicative of fructose degradation during fermentation with LAB (29, 119, 120), while 2-ethylmalate detected in beer is indicative of yeast fermentation (121). A significant increase in methionine following sourdough bread (52) and cheese consumption (59) is in line with previous reports of methionine detected in fermented foods, and methionine (and lysine) production by some cultures of *Lactobacillus* and yeasts used in the fermentation of cereals (122, 123).

For tea, an increase in theabrownins (phenolic pigment compounds) reflects the fermentation of catechins and gallate derivatives. Along with acting as a possible FIB for the fermentation process, theabrownins may serve a dual role as health biomarkers as well, as it was recently demonstrated that theabrownins from post-fermented pu-erh tea exerts cholesterol- and lipid-lowering effects via modulation of gut microbiota and bile acid metabolism (124). Furthermore, increased levels of gallic acid for tea and wine results from the fermentation of the polyphenol EGCG, which is a common food group-level metabolite for these foods (125, 126).

Similarly, as a major byproduct of alcoholic fermentation with yeast, ethanol and its metabolites (*e.g.*, ethyl glucuronide, ethyl sulfate) could be considered fermentation biomarkers for alcoholic beverages such as wine, beer, as well as distilled liquor (127). Ethanol has been widely used by food and forensic scientists alike to detect and monitor levels of alcohol, typically in blood or expired breath. However, ethanol was not increased in blood following consumption of Şalgam, boza, kimiz, or kefir, which have low alcoholic content due to mainly being fermented with LAB (128). As such, the utility of ethanol as a biomarker may not extend to low-alcohol beers, dealcoholized wine, or similar variations of these beverages, due to differences in the fermentation process (*e.g.*, selection of yeast strains that do not consume or produce ethanol) or the removal of alcohol from the fermented product.

LAB are used for the fermentation of many food substrates (129), and several classes of compounds are produced via lactic fermentation processes. During fermentation, LAB can convert amino acids into amine-containing compounds referred to as biogenic amines (130), which can be detected at fairly high concentrations in the final fermented foods. Fermented sausages, for example, have been reported to contain high concentrations of biogenic amines (spermine, spermidine) since their production is primarily attributed to the action of decarboxylase-positive bacteria and meat enzymes during fermentation and ripening (131). Biogenic amines serve a critical physiological role as precursors for the synthesis of hormones, alkaloids, nucleic acids, and proteins, act as neurotransmitters, and play a role in other central biological functions (132, 133). While accumulation of biogenic amines in the body has toxicological consequences (130), moderate levels are generally detoxified by amino oxidases in the gut. Despite extensive reports describing the presence of biogenic amines in fermented foods such as cheese, fermented vegetables, wine, and fermented meats, in the current search, only one study reported an increase in spermidine levels (fecal) following yoghurt consumption for 2 to 4 weeks (134, 135), and it is unknown whether this increase is a result of food consumption or synthesis by the gut microbiota. A review of biogenic amines in food products further indicates that biogenic amines are also naturally present in grapes, raw meat and seafood, and fresh milk (136), which offers an explanation of

why biogenic amines have not served a prominent role as FIBs for fermented foods. Additionally, biogenic amines are notoriously chemically unstable, as well as light- and pH-sensitive, which makes their analysis difficult (137). However, some research has indicated that further chemical reactions of indoleamines with acetaldehyde can produce novel metabolites during fermentation, ripening, and storage that could be more specific for fermented foods. In a study by Ohya *et al.* (138), 4-methylspinacemine and its metabolite, 1,4-dimethylspinacemine (Pictet-Spengler condensation reaction products of histamine with acetaldehyde), were increased in the urine of volunteers following consumption of soy sauce (with a meal) or Appenzeller cheese. Analysis of various fermented foodstuffs, including soy sauce, fish sauce, cheese, and shao hsing wine, confirmed the presence of both compounds in these foods (138).

A number of vitamins such as folate, vitamin B12, riboflavin and vitamin K are produced from fermentation of dairy products, elevating the nutritional quality of the product (7). In particular, many foods fermented using *B. subtilis* give rise to menaquinone 7 (MK-7, or vitamin K2), which is a long-chain menaquinone primarily synthesized by bacteria and detected abundantly in cheese, as well as fermented soybean products. However, MK-7 can also be synthesized by the gut microbiota, indicating a dual exogenous/endogenous origin of this compound (139). In the current search, increased MK-7 in serum or plasma was reported following consumption of the fermented soy product, natto (140-143), and validated in cross-sectional studies based on frequency of natto consumption (141, 143).

Indoles, metabolites derived from tryptophan which act as endogenous ligands for the aryl hydrocarbon receptor, are also known to be produced from LAB via the tryptophanase pathway (144). In the current review, indoles (especially indole-3-lactic acid) have been detected in biosamples following the consumption of multiple fermented foods, including yoghurt, cheese, beer, coffee, and bread. In addition, multiple strains of LAB produce phenyllactic and 4-hydroxyphenyllactic acids, and these metabolites have been shown to play a role in the quality and preservation of foods (145). D-phenyllactic acid was increased in serum and urine following the acute consumption of Gruyère cheese (59, 60) and in plasma and urine following the acute consumption of sauerkraut (96). Given that D-phenyllactic acid has also been confirmed to be present in other LAB-fermented foods including kimchi and sourdough (146-148), further investigation is warranted for this metabolite as a promising 'fermentation-dependent' FIB for lactic-fermented foods.

Heterogeneity of fermented foods and impact on FIBs

An inherent challenge in searching for FIBs of fermented foods is attributable to the heterogeneity of this food group. As evident in this review, virtually all food substrates can be fermented, and differences in fermentation conditions, such as type of microorganisms involved, duration of fermentation, even minute changes in environmental conditions, further contribute to producing foods with vastly different compositional profiles. To illustrate, consider the fermentation of milk to produce different types of cheeses. The common starting substrate, milk, can originate from cows, goats, sheep, water buffalo, or a combination of these (149). Some cheeses are ripened with internal (Grana Padano) or surface bacteria (Havarti, Limburger), others with internal molds (Roquefort) or surface (brie, camembert) moulds (149). Even within bacteria-ripened cheese, interestingly, the 'holes' in the cheese are created via different processes: in Swiss Emmental cheese by fermentation of lactate by *Propionibacterium freudenreichii*, and in Dutch Gouda cheese by fermentation of citrate by LAB (149).

Aside from the use of different microoganisms, the abundance of microbes can vary widely, and in some cases, the microorganisms are intentionally removed (*e.g.*, heat inactivation, filtration). Even in the absence of a heat or separation step, the number of microbes present at the time of consumption depends on multiple factors, such as the initial composition, storage conditions, and the age of the food (150). In a review by Rezac *et al.* (151), levels of live microorganisms in fermented foods were found to be dependent on geographical region and age of the food. For instance, microbial counts were undetectable ($<10^3$ cfu/g) in

Swiss Gruyère or Grana Padano cheeses aged greater than 1 year, while high counts (10^9 cfu/g) were found in Tilsit cheese aged for 2 to 4 months (151). Given that fermentation relies on the enzymatic activities of microorganisms to convert food components, the chances of fermentation-dependent metabolites being detected in biosamples to be identified as FIBs is inevitably linked to the amount of microorganism present in the food product. Furthermore, while industrial fermentations are typically conducted using predefined starter cultures to guarantee consistency, safety, as well as specific metabolic activities, artisanal fermentations (which are gaining in popularity) rely on mixed sources or microbes endogenous to the raw food (152). This further complicates the generalizability of any FIBs identified for industrially fermented foods, and necessitates careful documentation of fermentation procedures and metabolic products in all cases.

Impact of gut microbiota on FIBs for fermented foods

A second complexity in exploring FIBs for fermented foods involves the food-gut microbiota interface. Many fermented foods can act as a delivery vehicle for live microorganisms that can subsequently contribute to a changed gut microbiota landscape and altered metabolite appearance (8, 22). The diversity of microorganisms found in various fermented foods, as well as their functional properties, have been reviewed previously (22, 153). Both the gut and food microbiota can 'ferment' food components, and it has recently been documented that over 40% the LAB consumed via the ingestion of fermented foods (mainly cheese and other fermented milk products) become members of the gut microbiome (152). Interestingly, the species of LAB colonized in the gut was found to be regionally dependent, with *S. thermophiles* and lactobacilli linked to yoghurt and dairy product consumption in Western diets, and heterofermentative *Leuconostoc* and *Weissela* linked to fermented vegetables and cereal-based foods predominant in non-Western diets (152). A further study by Taylor *et al.* (154) indicated that gut microbiome composition and functional profile are also affected by the frequency of consumption of fermented plants, with several microbes (*L. brevis, L. kefiranofaciens, L. parabuchneri, L. helveticus*, and *L. sakei*) associated with both fermented foods and self-reported 'consumers', but not 'non-consumers' (154). Furthermore, while transient or long-term intake of fermented foods may differentially impact the gut microbiome, and the response of the microbiome to diet remains highly personalized (155). Collectively, these reports reflect the challenge in delineating the origin of a FIB as from a fermented food, or from a non-fermented food transformed by the gut microbiota.

Representation of fermented foods consumed globally in the literature

While our goal was to search for FIBs for fermented food products consumed globally, a small number of fermented food products were represented in the current literature. To-date, the majority of research has concentrated on coffee, beer, wine, chocolate, bread, and fermented dairy products, as described above. Studies on fermented foods consumed in large quantities in Africa and Asia, for example products from rice (idli, dosa, dhokla), corn (ogi, kenkey, pozol), or sorghum (injera, kisra), fermented alcoholic beverages (sake, bouza, chichi, mahewu, boza) (3-5), were not identified, indicating a gap in the scientific literature. There exists a great opportunity for the further exploration and validation of biomarkers for less-commonly investigated fermented foods, the results of which will help benefit fermentation-dependent FIBs for fermented foods overall.

Conclusions

The large number of different food-level, food group-level, and fermentation-dependent compounds identified in this literature search may be promising FIBs for fermented food products if combined in a multi-marker approach, and needs to be validated in free-living cohorts with uncontrolled diets. While fairly specific food-level and food group-level biomarkers exist for commonly consumed fermented foods (*e.g.*, trigonelline for coffee, pentadecanoic acid for dairy), this review captured several fermentation-dependent FIBs common

among foods fermented by the same fermentation process (*e.g.*, ethanol and metabolites for alcoholic fermentation, methionine, indoles, and 3-phenyllactic acid from lactic-fermentation). Further, several gaps in the literature were revealed, particularly in the lack of studies on FIBs for fermented meats, fish, fruits, and vegetables, which presents an opportunity for future scientific investigation. Expanding the repertoire of FIBs for different fermented food products will greatly aid epidemiological efforts aimed to associate fermented foods with various health outcomes.

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List of abbreviations

ACE-1, angiotensin-1-converting enzyme; FIB, food intake biomarker; FFQ, food frequency questionnaire; LAB, lactic acid bacteria.

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Supplementary Materials

Table S1. Food-specific keywords used in the literature search for candidate biomarkers of fermented food intake			
Operator	**Database**	**Field**	**Keywords**
General Fermented Foods			
AND	Pubmed	Title/Abstract	ferment* OR "fermented food*" OR "fermented product*"
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
Fermented Dairy			
AND	Pubmed	Title/Abstract	cheese OR yoghurt OR yogurt OR yoghourt OR yakult OR "creme fraiche" OR quark OR kefir OR lassi OR "sour cream" OR "soured milk" OR "cultured milk" OR "cultured dairy" OR "fermented dairy" OR "fermented milk" OR buttermilk
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
NOT	Pubmed	Title/Abstract	"breast milk" OR "breast feeding" OR bone OR muscle OR allerg* OR "phyto ster*" OR "plant ster*" OR phytoster* OR newborn* OR infant*
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
Fermented Meat & Fish			
AND	Pubmed	Title/Abstract	"fermented meat" OR salami OR pepperoni OR chorizo OR cervelat OR mettwurst OR "summer sausage" OR sucuk OR "fermented sausage" OR "cured meat" OR "dried meat" OR "dry sausages" OR "dried sausages" OR "processed meat" OR "fermented fish" OR "fish sauce" OR "shrimp paste" OR "shrimp sauce"
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
Fermented Fruits & Vegetables			
AND	Pubmed	Title/Abstract	"fermented vegetable*" OR "fermented fruit*" OR sauerkraut OR olive* OR pickle* OR "fermented cucumber*" OR kimchi OR paocai
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
NOT	Pubmed	Title/Abstract	oil* OR "olive leaf extract"
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
Fermented Legumes (Including Soy)			
AND	Pubmed	Title/Abstract	"fermented soy*" OR "fermented bean" OR "soy sauce" OR "soya sauce" OR "soybean paste" OR miso OR tempeh OR natto OR cheonggukjang OR doenjang OR doubanjiang OR douchi OR gochujang
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
NOT	Pubmed	Title/Abstract	milk OR allerg*
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
Fermented Cereals & Grains			
AND	Pubmed	Title/Abstract	"fermented cereal*" OR "fermented grain*" OR "fermented wheat" OR "fermented oat*" OR "fermented rice" OR bread* OR sourdough OR crispbread
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
Fermented Beverages			
AND	Pubmed	Title/Abstract	"fermented beverage*" OR "fermented drink*" OR beer OR wine OR cider OR kombucha OR pulque OR coffee OR "fermented tea" OR "dark tea" OR "yellow tea" OR puer OR pu'er OR pu-er* OR fuzhuan OR "fu zhuan"
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
NOT	Pubmed	Title/Abstract	poison* OR drug* OR smoking OR toxic*
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
Other			
AND	Pubmed	Title/Abstract	chocolate OR cocoa OR "fermented condiment" OR "vinegar" OR tabasco OR worcestershire OR worcester
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	

Table S2. Operators used in the literature search for candidate biomarkers of fermented food intake			
Operator	**Database**	**Field**	**Keywords**
AND	Pubmed	All fields	biomarker* OR marker* OR metabolite* OR biokinetics OR biotransformation OR pharmacokinetics
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
AND	Pubmed	All fields	intake* OR meal* OR diet* OR ingestion OR consumption OR eat* OR drink* OR administration
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
AND	Pubmed	All fields	human* OR men OR women OR patient* OR volunteer* OR participant* OR individual* OR subject*
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	
AND	Pubmed	All fields	urine OR plasma OR blood OR serum OR excretion OR tissue* OR faeces OR feces OR “fecal water” OR “faecal water” OR nail* OR hair
	Web of Science	Topic	
	Scopus	Article Title/Abstract/Keywords	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cocoa	New Nordic Diet vs. Average Danish Diet	15±7 g/day (derived from 3-day weighted food records)	6-month, randomized, controlled, parallel	107 healthy volunteers	U(H)PLC-QTOF-MS	Urine	• 7-Methyluric acid • AMMU • Theobromine	(1)
Cocoa	New Nordic Diet vs. Average Danish Diet	Exact intakes not reported (derived from 3-day weighted food records)	6 month, randomized, controlled, parallel; 3-day weighted food records (24-h)	161 healthy adults	U(H)PLC-QTOF-MS	Urine	• **3,7-Dimethyluric acid** • **7-Methyluric acid** • **7-Methylxanthine** • **AMMU** • **Theobromine**	(2)
Cocoa	Cocoa or derived products	≥3x30 g/week chocholate and/or cocoa powder	Cross-sectional	64 healthy adults	HPLC-QTOF-MS	Urine	• **(Epi)catechin glucuronide** • **(Epi)catechin sulfate** • **3,7-Dimethyluric acid** • **3-Methyluric acid** • **3-Methylxanthine** • **4-Hydroxy-5-(dihydroxyphenyl) valeric acid** • **4-Hydroxy-5-(dihydroxyphenyl) valeric acid glucuronide** • **4-Hydroxy-5-(dihydroxyphenyl) valeric acid sulfate** • **4-Hydroxy-5-(hydroxy-methoxyphenyl) valeric acid sulfate** • **4-Hydroxy-5-(hydroxyphenyl) valeric acid sulfate** • **4-Hydroxy-5-(hydroxyphenyl) valeric acid sulfate** • **4-Hydroxy-5-(phenyl) valeric acid sulfate** • **7-Methylxanthine** • **AMMU** • **AMMU isomer** • **Aspartyl-phenylalanine** • **Cyclo(aspartylphenylalanyl)** • **DHPV sulfoglucuronide** • **DHPV glucuronide** • **DHPV sulfate** • **Furoylglycine** • **Hydroxyphenyl-valerolactone glucuronide** • **Hydroxyphenyl-valerolactone sulfate** • **MHPV** • **MHPV glucuronide** • **Theobromine** • **Vanillic acid** • **Vanillin sulfate** • **Xanthine**	(3)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cocoa	Cocoa powder in water vs. cocoa powder in milk	40 g/day + 250 mL water or milk	Acute, randomized, controlled, crossover	10 healthy adults	HPLC-QTOF	Urine	• **3,5-Diethyl-2-methylpyrazine** • **3,7-Dimethyluric acid** • **3-Methyluric acid** • **3-Methylxanthine** • **4-hydroxy-5-(3,4-dihydroxyphenyl) valeric acid** • **7-Methyluric acid** • **7-Methylxanthine** • **AMMU** • **Caffeine** • **Cyclo(Pro-Pro)** • **Cyclo(Ser-Tyr)** • **DHPV glucuronide** • **DHPV sulfate** • **Epicatechin-O-sulfate** • **Hydroxyacetophenone** • **Hydroxynicotinic acid** • **MHPV** • **MHPV glucuronide** • **O-Methylepicatechin** • **Theobromine** • **Trigonelline** • **Tyrosine** • **Tyrosine** • **Vanillic acid** • **Vanilloylglycine**	(4)
Cocoa	Cocoa powder in milk vs. milk only	40 g/day + 500 mL skimmed milk	8-week, randomized, controlled, crossover	20 patients at high risk of CVD	HPLC-QTOF	Urine	• **(Epi)catechin glucuronide** • **N-[4-hydroxy-3 -methoxy-E-cinnamoyl]-L-aspartic acid** • **N-[4-hydroxycinnamoyl]-L-aspartic acid** • **Theobromine** • **Vanillic acid glucuronide** • **Vanillic acid sulfoglucuronide** • **Vanilloylglycine** • **3,5-Diethyl-2-methylpyrazine** • **3,7-Dimethyluric acid** • **3-Methyluric acid** • **3-Methylxanthine** • **4-Hydroxy-5-(dihydroxyphenyl) valeric acid glucuronide** • **4-Hydroxy-5-(dihydroxyphenyl) valeric acid sulfate** • **4-Hydroxy-5-(hydroxy-methoxyphenyl) valeric acid glucuronide** • **7-Methyluric acid**	(5)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
							• **7-Methylxanthine** • **AMMU** • **Cyclo(propylalanyl)** • **DHPV glucuronide** • **DHPV sulfoglucuronide** • **DHPV sulfate** • **Epicatechin sulfoglucuronide** • **Hydroxyphenyl-γ-valerolactone glucuronide** • **Methyl(epi)catechin sulfate** • **MHPV** • **MHPV sulfate**	
Cocoa	Cocoa powder in milk	40 g/day + 250 mL milk	Acute intervention	10 healthy adults	HPLC-QTOF-MS	Urine	• 3-Methylxanthine • Theobromine • Vanilloylglycine • Xanthurenic acid • 7-Methylxanthine • DHPV glucuronide • Furoylglycine • **N-methylguanine**	(6)
Cocoa	Chocolate (Flavan-3-ol-enriched dark, standard dark, white)	60 g + 400 mL still table water (200 mL in TO 2h and 4h)	Acute, randomized, controlled, crossover	42 healthy adults	NMR	Urine	• **2-Hydroxyisobutyrate** • **3-Hydroxyisobutyrate** • **3-Hydroxyisobutyrate** • **4-Hydroxyphenylacetate** • **Arginine** • **Creatinine** • **Alanine** • **Dimethylamine** • **Glycine** • **Lactate** • **N-acetylated compounds** • **N-methylnicotinamide** • **Pyruvate** • **Theobromine** • **Tyrosine** • **Valine** • **Epicatechin derivative** • **Methylxanthines**	(7)
					LC-MS		• **Caffeine** • **DHPV glucuronide** • **DHPV sulfate** • **Epicatechin-O-sulfate** • **Hydroxynicotinic acid**	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• **MHPV glucuronide** • **O-feruloylquinate** • **Vanilloylglycine** • **Epicatechin derivative** • **Methylxanthines** • **3,7-Dimethylurate** • **4-hydroxy-5-(3,4-dihydroxyphenyl) valeric acid** • **7- and 3-methyluric acid** • **7- and 3-methylxanthine** • **AMMU**	
Cocoa, wine	Chocolate	Habitual intake (dose not reported); assessed by FFQ	Prospective cohort	3559 adult female twins	U(H)PLC-MS/MS	Serum/plasma	• **7-Methylxanthine** • **Theobromine** • **1-Methylxanthine** • **3-Hydroxypyridine sulfate** • **3-Methyl catechol sulfate** • **Catechol sulfate** • **Cyclo(leu-pro)** • **O-methylcatechol sulfate** • **Quinate**	(8)
	Wine	Habitual intake (dose not reported); assessed by FFQ					• **1-docosahexaenoylglycerophospho ethanolamine** • **2-aminobutyrate** • **2-hydroxybutyrate** • **2-Hydroxyisovalerate** • **3-(4-hydroxyphenyl) lactate** • **3-Methyl-2-oxobutyrate** • **4-androsten-3beta,17beta-diol disulfate** • **4-Methyl-2-oxopentanoate** • **5-alpha-androstan-3beta-17beta-diol disulfate** • **Arachidonate (20:4n6)** • **Benzoate** • **beta-hydroxyisovalerate** • **Caprate (10:0)** • **Caprylate (8:0)** • **Docosahexaenoate (22:6n3)** • **Docosapentaenoate (22:5n3)** • **Eicosapentaenoate (20:5n3)** • **Epiandrosterone sulfate** • **myo-Inositol** • **Phosphatidylcholine diacyl C32:1** • **Phosphatidylcholine diacyl C36:5** • **Pipecolate** • **Piperine**	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• **Scyllo-inositol** • **Stearidonate (18:4n3)** • **Theophylline**	
Cocoa	Chocolate (80% cocoa)	100 or 200 g/day	2-day, randomized, controlled	15 heathy young adults	U(H)PLC-ESI-QTOF-MS	Urine	• Epicatechin-O-sulfate • Epicatechin-O-glucuronide • O-Methyl-epicatethin-O-sulfate • O-Methyl-epicatethin-O-glucuronide • O-Methyl-epicatethin-sulfate-O-glucuronide • O-Methyl-epicatethin-disulfate-O-glucuronide • Epicatechin-O-glucoside • 5-(Hydroxyphenyl)-g-valerolactone-O-glucuronide • 5-(Hydroxyphenyl)-g-valerolactone-O-glucuronide • 5-Phenyl-g-valerolactone-glucuronide • 5-Phenyl-g-valerolactone-methoxy-glucuronide • 5-(Tri hydroxyphenyl)-g-valerolactone-glucuronide • 5-(Hydroxy phenyl)-g-valerolactone-methoxy-sulfate • 5-(Dihydroxy phenyl)-g-valerolactone-sulfate • 5-Phenyl-g-valerolactone-methoxy-sulfate • 5-(Hydroxyphenyl)-g-valerolactone-sulfate • 5-Phenyl-g-valerolactone-sulfate • 5-(Dihydroxy phenyl)-g-valero lactone	(9)
Cocoa	Dark chocolate (74% cocoa) consumption in volunteers with different anxiety traits	40 g/day	2-week, randomized, parallel, open	30 healthy volunteers	NMR, GC-MS, LC-MS/MS	Plasma, urine	• Asparagine • Corticosterone • Cortisol • Cystine • 4-Hydroxyphenylacetate • Adrenaline • Glucose-6-phosphate • Normetanephrine • Threonic acid • Phenylacetylglutamine • p-Cresol sulfate • (Significant in urine, but not plasma; dark chcolate consumption further modulated metabolites associated with anxiety traits)	(10)
Cocoa	Chocolate consumption between 'chocolate desiring' vs. 'chocolate indifferent' individuals	50 g	5-day, double crossover	22 healthy men	NMR	Urine	• **2-hydroxyhippurate** • **2-Hydroxyisobutyrate** • **4-cresol** • **Acetone** • **Carnitine** • **Isobutyrate** • **Methylsuccinate** • **N-acetyl-carnitine**	(11)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• **Taurine** • **Trigonelline** • **Trimethylamine** • 3-Hydroxyisobutyrate • 4-Hydroxyphenylacetate • Acetoacetate • Citrate • Dimethylglycine • Glycine • Phenylacetylglutamine	
Cocoa, coffee, wine	Chocolate candies	Habitual intake; dose not reported (from FFQ)	Nested case-control	1369 premenopausal women	U(H)PLC-MS/MS	Serum	• **3,7-Dimethylurate** • **3-Methylxanthine** • **7-Methylurate** • **7-Methylxanthine** • **Theobromine**	(12)
	Coffee, caffeinated	Habitual intake; dose not reported (from FFQ)					• **1,3,7-Trimethylurate** • **1,3-Dimethylurate** • **1,7-Dimethylurate** • **1-Methylurate** • **1-Methylxanthine** • **AAMU** • **Caffeine** • **Paraxanthine** • **Theophylline**	
	Coffee, decaffeinated	Habitual intake; dose not reported (from FFQ)					• **2,3-dihydroxypyridine**	
	Red wine	Habitual intake; dose not reported (from FFQ)					• **2,3-Dihydroxyisovalerate** • **Ethyl glucuronide**	
	Total coffee	Habitual intake; dose not reported (from FFQ)					• **3-Hydroxypyridine sulfate** • **3-Methyl catechol sulfate** • **Citraconate/glutaconate** • **Quinate** • **Trigonelline**	
	Total wine	Habitual intake; dose not reported (from FFQ)					• **2,3-Dihydroxyisovalerate** • **Androstenediol (3β,17β) monosulfate** • **3-Carboxy-4-methyl-5-propyl-2-furanpropanoic acid** • **Ethyl glucuronide** • **Oleoyl-linoleoyl-glycerol (18:1/18:2)** • **Sphingomyelin (d18:2/18:1)**	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
	White wine	Habitual intake; dose not reported (from FFQ)					• **2,3-Dihydroxyisovalerate** • **Ethyl glucuronide**	
Bread	Wholegrain sourdough rye at different doses	0 g/day, 48 g/day, 96 g/day	4-week, randomized, controlled, crossover	33 healthy adults	FIE-MS	Urine	• **HBOA glucuronide** • **HHPAA glucuronide** • **HHPAA sulfate** • **C13H21O3 glucuronide** • **C14H25O4 glucuronide** • **Creatinine** • **HPAA sulfate** • **N-feruloylglycine sulfate** • **Phenylacetylglutamine derivative**	(13)
Bread	Sourdough fermented endosperm rye vs. white wheat bread	110.6 g (sourdough); 105.9 g (white wheat bread)	Acute, randomized, controlled, crossover	16 healthy adults	GCxGC-TOF-MS	Plasma	Sourdough: • **2,4-Dihydroxybutanoic acid** • **2-Oxo-butanoic acid** • **Alpha-ketoglutaric acid** • **Benzeneacetic acid** • **Citrate** • **Lysine** • **Methionine** • **Norvaline** • **Phenylalanine** • **Propanedioic acid** • **Ribitol** • **Threonic acid** White wheat bread: • **2-(Z)-Butenedioic acid** • **2,4-Dihydroxybutanoic acid** • **2,8-Dihydroxyquinoline glucuronide** • **2-Oxo-butanoic acid** • **Alpha-ketoglutaric acid** • **Ascorbic acid** • **Benzeneacetic acid** • **Hydrocaffeic acid** • **Lysine** • **Norleucine** • **Picolinic acid** • **Propanedioic acid** • **Succinic acid**	(14)
Bread	White bread vs. non-consumers	>1 portion/day (habitual intake)	Multi-centre, randomized,	255 healthy adults	HPLC-QTOF-MS	Urine	• **2-Aminophenol sulfate** • **2-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one**	(15)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
			controlled, parallel				• **2-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one glucuronide** • **DHPPA glucuronide** • **Hydroxybenzoic acid glucuronide** • **HPAA glucuronide** • **Riboflavin**	
	Wholegrain bread vs. non-consumers	>1 portion/day (habitual intake)					• **2,8-Dihydroxyquinoline glucuronide** • **2-Aminophenol sulfate** • **HBOA glycoside** • **2-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one** • **2-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one glucuronide** • **HHPAA** • **3,5-Dihydroxyphenylethanol sulfate** • **3-Indolecarboxylic acid glucuronide** • **DHPPA glucuronide** • **DHPPTA sulfate** • **Dihydroferulic acid sulfate** • **Enterolactone glucuronide** • **Hydroxybenzoic acid glucuronide** • **HPAA glucuronide** • **Pyrraline** • **Riboflavin**	
Bread	Whole grain bread replacement and high fish and bilberry consumption vs. wholegrain replacement and normal fish and berry consumption vs. avoidance of wholegrain cereals, fish, and berries	20-25% of total daily energy intake	12-week, randomized, controlled, parallel	106 healthy adults	U(H)PLC-QTOF-MS	Plasma	• AR 21:1-Gln • AR 19:0-Gln • Gamma-Butyrobetaine Pipecolic acid betaine	(16)
Bread	Rye bread vs. wheat bread	214 g/day (rye); 179 g/day (wheat)	8 week, randomized, controlled, crossover	39 postmenopausal women	GCxGC-TOF-MS	Plasma	Rye bread: • **Campesterol** • **Dodecanamide** • **Indole-3-acetic acid** • **Inosose**	(17)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
							• **myo-Inositol** • **Ribitol** • **Ribonic acid** • **Silanamine** • **Sitosterol** • **Trimethylsiloxy proline** Wheat bread: • **1-Monooleoylglycerol trimethylsilyl ether** • **4-[39-(Triethylsilyl)propyl]phenol** • **Butanoic acid** • **d-Erythrotetrofuranose** • **Glutamic acid** • **N-Methyl-N-(2,4,6-trimethylphenyl)formamide** • **Palmitic acid** • **Pyrrole-2,5-dione trimethylsilate** • **Tyrosine** • **Urate**	
Bread	Wholegrain rye vs. white wheat bread enriched with fermented rye bran	6-10 slices/day	4-week, randomized, controlled, crossover	15 healthy adults	U(H)PLC-QTOF-MS	Plasma	• **AR C17-glucuronide** • **AR C17-sulfate** • **AR C19:0-glucuronide** • **AR C19:0-sulfate** • **AR C19:1-glucuronide** • **AR C19:1-sulfate** • **AR C21:0-glucuronide** • **AR C21:1-glucuronide** • **AR C21:1-sulfate** • **AR C21:2-glucuronide** • **AR C23:2-glucuronide** • **3,5-Dihydrobenzoic acid** • **2-Aminophenol sulfate** **Pipecolic acid betaine**	(18)
Bread	Rye bran wheat bread vs. wholegrain wheat bread	196 g (rye grain); 208 g (wholegrain)	Acute crossover	12 healthy volunteers	UPLC-MS/MS (untargeted)	Urine	• Cysteine • N-acetylcysteine • Indolelactate • 4-Acetamidobutanoate • Imidazole lactate • trans-4-Hydroxyproline • N-Acetylarginine • N2,N5-Diacetylornithine • Argininosuccinate • Creatinine • 5-Hydroxyindoleacetate • Xanthurenate	(19)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• Dopamine sulfate • N-Acetylputrescine • Cysteinylglycine • Phenylacetylglutamine • Citramalate • Pseudouridine • 4-Ureidobutyrate • Uridine • 5,6-Dihydrouracil • Thymine • 3-Methylcytidine • 7-Methylguanine • N2,N2-Dimethylguanosine • N1-Methyladenosine • N6-Carbamoylthreonyladenosine • Adenosine • Adenine • Urate • Azelate • Pimelate • Dimethylmalonic acid • 2-Aminooctanoate • Ribitol • Ribulose/xylulose • N1-Methyl-2-pyridone-5-carboxamide • 1-Methylnicotinamide • Oxalate • Citraconate/glutaconate • **Tartarate** • **Vanillic acid** • **3,5-DHBA** • **Ferulic acid 4-sulfate** • **Syringic acid** • **2,3-Dihydroxyisovalerate** • **2-Oxindole-3-acetate** • **Gentisic acid-5-glucoside** • **4-Vinylguaiacol sulfate** • **1,2,3-Benzenetriol sulfate** • **3-Hydroxypyridine sulfate** • **1,2,3-Benzenetriol sulfate** • Lanthionine • Sulfate • HPAA sulfate	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
							• 4-Acetylphenol sulfate • 3-Methylcatechol sulfate • 3-Methylcatechol sulfate • 3-Methoxycatechol sulfate • 1-Methylxanthine • 1-Methylurate	
					HPLC-MS/MS (Targeted)		• 3,5-DHBA • 3,5-DHPPTA • 3,5-DHBA glycine • 3,5-DHBA sulfate • 3,5-DHPPA sulfate • 3,5-DHPPTA sulfate • 3,5-DHPHTA sulfate • 2-(3,5-dihydroxyphenyl)ethanol sulfate • HPAA • HPAA sulfate • 2-Aminophenol sulfate • HHPAA sulfate • HHPPA sulfate • Isopropyl 2hydroxyphenylcarbamate • Ferulic acid sulfate • Caffeic acid sulfate • Vanillic acid sulfate • Homovanillic acid sulfate • DHFA sulfate • Feruloyglycine • Feruloylglycine sulfate • Glycochenodeoxycholic acid • Glycochenodeoxychol-5en-24-oic acid • Glycochenodeoxycholic acid glucuronide • Glycochenodeoxychol-5en-24-oic acid glucuronide • Enterolactone glucuronide	
Bread	Wholegrain sourdough bread vs. white wheat bread enriched with native unprocessed rye bran vs. white wheat bread enriched with bioprocessed	109 to 166 g (standardised to 50 g carbohydrates and 20 g fiber)	Acute, randomized, crossover	12 healthy volunteers	LC-QTOF-MS and MS/MS	Plasma	• Benzoxazinoid-derived phenylacetamide sulfates (hydroxy-N-(2-hydroxyphenyl) acetamine, and N-(2-hydroxyphenyl) acetamide) (Fermentation has a central role in modulating the phytochemical profile of the breads)	(20)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	rye bran vs. white wheat bread control							
Bread	Wholegrain bread vs. wholemeal rye bread	Advised to take test products instead of customarily used breads and baked products	4-week, randomized, crossover	20 volunteers with slightly elevated serum cholesterol	UPLC-QTOF-MS	Urine	• 3,5-Dihydroxyhydrocinamic acid sulfate • Non-identified nitrogen containing metabolite • Ascorbic acid • Non-identified nitrogen containing metabolite • 2-Aminophenol sulfate • Non-identified nitrogen containing metabolite • Nonanedioic acid • DHPPA glucuronide • Indolylacryloylglycine • Enterolactone glucuronide • DHPPA sulfate • Non-identified nitrogen containing metabolite • Ferulic acid-4-O-sulfate • 2,4-Dihydroxy-1,4-benzoxazin3-one sulfate • 3,5-Dihydroxyphenylethanol sulfate • 1,3,4,5Tetrahydroxycyclohexane-1carboxylic acid	(21)
Coffee	Coffee, lower intake vs. Coffee, higher intake	4 cups/day (600 mL/day) lower intake; 8 cups/day (1200mL/day) higher intake	1-month, single-blind, crossover	47 healthy adults	U(H)PLC-ESI-MS/MS	Serum	Lower intake: • **Creatinine** • **Guanidinoacetate** • **Imidazole lactate** • **Isovalerylcarnitine** • **Cysteine** • **4-Acetamidobutanoate** • **N-acetylputrescine** • **Indole-3-lactic acid** • **Kynurenine** • **2-Hydroxyphenylacetate** • **Glucuronate** • **Trigonelline** • **Citraconate/glutaconate** • **4-Androsten-3beta,17beta-diol monosulfate** • **Epiandrosterone sulfate** • **Etiocholanolone glucuronide** • **Urate** • **7-Methylguanine** • **N-acetylcarnosine** • **DSGEGDFXAEGGGVR*** • **3-(3-hydroxyphenyl) propionate** • **3-(3-hydroxyphenyl) propionate sulfate** • **3-Hydroxyhippurate**	(22)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
							• **3-Methyl catechol sulfate** • **3-Phenylpropionate** • **4-Vinylphenol sulfate** • **Catechol sulfate** • **Hippurate** • **O-methylcatechol sulfate** • **3-Hydroxypyridine sulfate** • **N-methylpipecolate** • **Cinnamoylglycine** • **Dihydroferulic acid** • **Homostachydrine** • **N-(2-furoyl)glycine** • **Pyrraline** • **Quinate** • **1,3,7-Trimethylurate** • **1,3-Dimethylurate** • **1,7-Dimethylurate** • **1-Methylurate** • **1-Methylxanthine** • **3,7-Dimethylurate** • **3-Methylxanthine** • **AAMU** • **7-Methylxanthine** • **Caffeic acid sulfate** • **Caffeine** • **Paraxanthine** • **Theobromine** • **Theophylline** Higher intake: • **Creatinine** • **Guanidinoacetate** • **Imidazole lactate** • **Isovalerylcarnitine** • **Cysteine** • **4-Acetamidobutanoate** • **N-acetylputrescine** • **Indole-3-lactic acid** • **Kynurenine** • **2-Hydroxyphenylacetate** • **Glucuronate** • **Trigonelline** • **Citraconate/glutaconate** • **4-Androsten-3beta,17beta-diol monosulfate**	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• Epiandrosterone sulfate • Etiocholanolone glucuronide • Urate • 7-Methylguanine • N-acetylcarnosine • DSGEGDFXAEGGGVR • 3-(3-hydroxyphenyl) propionate • 3-(3-hydroxyphenyl) propionate sulfate • 3-Hydroxyhippurate • 3-Methyl catechol sulfate • 3-Phenylpropionate • 4-Vinylphenol sulfate • Catechol sulfate • Hippurate • O-methylcatechol sulfate • 3-Hydroxypyridine sulfate • N-methylpipecolate • Cinnamoylglycine • Dihydroferulic acid • Homostachydrine • N-(2-furoyl)glycine • Pyrraline • Quinate • 1,3,7-Trimethylurate • 1,3-Dimethylurate • 1,7-Dimethylurate • 1-Methylurate • 1-Methylxanthine • 3,7-Dimethylurate • 3-Methylxanthine • AAMU • 7-Methylxanthine • Caffeic acid sulfate • Caffeine • Paraxanthine • Theobromine • Theophylline • Campesterol • N6-carbamoylthreonyladenosine • Palmitoyl dihydrosphingomyelin • Phosphate	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Coffee	Coffee, high consumption vs. coffee low consumption	1546 g/day (high); 337 g/day (low) (from FFQ)	Nested case-control	489 healthy adults	LC-MS and GC-MS	Serum	• **1,3,7-Trimethylurate** • **1,7-Dimethylurate** • **1-Methylurate** • **1-Methylxanthine** • **3-(3-hydroxyphenyl) propionate** • **3-Hydroxyhippurate** • **4-Vinylphenol sulfate** • **Caffeine** • **Catechol sulfate** • **Cinnamoylglycine** • **Cyclo(leu-pro)** • **N-(2-furoyl)glycine** • **Paraxanthine** • **Quinate** • **Theobromine** • **Theophylline** • **Trigonelline**	(23)
Coffee	Coffee	200 mL	Acute intervention	8 healthy adults	NMR	Urine	• **2-Furoylglycine** • **N-methylpyridinium**	(24)
Coffee	Coffee	350 mL	Acute intervention	9 healthy adults	HILIC-U(H)PLC-TOF-MS	Urine	• **N-methylpyridinium** • **Trigonelline** • **1,3- or 1,7-dimethyluric acid** • **3-Methylxanthine** • **7-Methylxanthine** • **Caffeine** • **Catechol sulfate** • **Dihydrocaffeic acid sulfate** • **Dihydroferulic acid** • **Dihydroferulic acid glucuronide** • **Dihydroferulic acid sulfate** • **Ferulic acid** • **Ferulic acid glucuronide** • **Ferulic acid sulfate** • **Guaiacol sulfate** • **N-feruloylglycine** • **Paraxanthine** • **Theobromine** • **Theophylline** • **Trigonelline**	(25)
Coffee	Coffee, instant	400 mL	Acute, randomized, controlled, crossover	9 healthy adults	U(H)PLC-MS	Plasma	• 4-Feruloylquinic acid • 4-Feruloylquinic acid lactone • Caffeic acid 3-O-sulfate	(26)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• Caffeic acid 4-O-sulfate • Caffeoylquinic acid lactone O-sulfates • Dihydrocaffeic acid • Dihydrocaffeic acid 3-O-glucuronide • Dihydrocaffeic acid 3-O-sulfate • Dihydrocaffeic acid 4-O-sulfate • Dihydrodihydrodimethoxycinnamic acid • Dihydroferulic acid • Dihydroferulic acid 4-O-sulfate • Dihydroisoferulic acid • Dihydroisoferulic acid 3-O-sulfate • Dihydroisoferulic acid O-glucuronide • Dimethoxycinnamic acid • Ferulic acid • (*iso*)Ferulic acid • (*iso*)Ferulic acid 3-O-glucuronide • (*iso*)Ferulic acid 3-O-sulfate • Ferulic acid 4-O-glucuronide • Ferulic acid 4-O-sulfate • 3-Feruloylquinic acid • 5-Feruloylquinic acid • 3-Feruloylquinic acid lactone • Feruloylquinic acid lactone O-glucuronide • Feruloylquinic acid lactone O-sulfate • m-CoA O-sulfate • o-CoA O-sulfate p-DHCoAO-sulfate	
Coffee	High coffee consumption vs. low coffee consumption	183 to 540 mL/day (high); 0 mL/day (low) (from FFQ, multiple 24-h recalls)	Prospective cohort	39 healthy adults (20 high consumers; 19 low consumers)	U(H)PLC-QTOF-MS	Urine	• **1,3- or 3,7-Dimethyluric acid** • **1,7-Dimethyluric acid** • **1-Methyluric acid** • **1-Methylxanthine** • **3-Hydroxyhippuric acid** • **AFMU** • **Atractyligenin glucuronide** • **Caffeine** • **Cyclo(isoleucyl-prolyl)** • **Dimethylxanthine (paraxanthine or theophylline) glucuronide** • **Hippurate** • **Kahweol oxide glucuronide** • **Kahweol oxide glucuronide analogue** • **Paraxanthine** • **Trigonelline**	(27)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• **Trimethyluric acid**	
Coffee	Coffee	4 cups/day; 8 cups/day	1-month controlled, crossover	47 healthy adults, habitual coffee drinkers	IMS-MS (lipidomics)	Serum	Low-dose: • TAG47:1-FA17:0 • TAG52:5-FA20:5 • PC(18:0/16:1) • PC(18:0/18:3) • PC(18:0/20:2) • PE(O-16:0/18:2) • PE(P-16:0/18:2) • PE(P-18:0/18:2) • DCER(24:0) High-dose: • **TAG47:1-FA17:0** • **TAG52:5-FA20:5** • **TAG60:11-FA22:5** • **PC(18:0/16:1)** • **PC(18:0/18:3)** • **PC(18:0/20:2)** • **PC(18:0/20:3)** • **PE(18:0/20:1)** • **PE(O-16:0/18:2)** • **PE(O-18:0/20:3)** • **PE(P-16:0/18:2)** • **PE(P-18:0/18:2)** • **DCER(24:0)** • **LCER(26:1)**	(28)
Coffee	Coffee consumers vs. non-consumers	Consumers (mean 506.4 g/day for women, 526.4 g/day for men); Non-consumers (0 g/day)	Cross-sectional, KarMeN study	48 healthy adults (consumers); 49 healthy adults (non-consumers)	HS-SPME-GCxGC-MS	Urine	• **3,4-Dimethyl-2,5-furadione** • **2-Methyl-furan** • **Guaicol** • **2-Methyl-butanoic acid** • **3-Methyl-butanoic acid** • **2-Vinylfuran**	(29)
Coffee	Total coffee	Consumers (≥50 mL/day)	Cross-sectional, PREDIMED study	1379 adults with T2D or CV risk factors; 285 non-coffee consumers	LC-MS/MS, U(H)PLC-Exactive Plus orbitrap MS	Plasma	• **Sphingomyelin (C24:0)** • **Caffeine** • **AAMU** • **Cotinine**	(30)
	Caffeinated coffee	Consumers (≥50 mL/day)		512 adults with T2D or CV risk factors			• **Sphingomyelin (C24:0)** • **Caffeine** • **AAMU** • **Cotinine**	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	Decaffeinated coffee	Consumers (≥50 mL/day)		721 adults with T2D or CV risk factors			• **Alpha-glycerophosphate** • **Hydroxyhippurate** • **Hippurate** • **Sphingomyelin (C24:0)** • **Phosphatidylcholine (C40:6)**	
Coffee	Habitual coffee intake	253 mL/day (France); 437 mL/day (Germany); 154 mL/day (Greece); 99 mL/day (Italy)	EPIC study (France, Germany, Italy, Greece)	451 participants from the EPIC cohort	U(H)PLC-MS/MS	Serum	• **Trigonelline** • **Caffeine** • **Paraxanthine** • **AAMU** • **Quinic acid** • **Cyclo(prolyl)-valyl** • **Cyclo(isoleucyl)-prolyl** • **Cyclo(leucyl)-prolyl** • **Pyrocatechol sulfate**	(31)
Coffee, multiple foods	Habitual intake of 58 different foods based on FFQ	<1X/week, 1X/week, 2-4X/week, 5-6X/week, 1X/day, >1X/day	Prospective cohort	68 volunteers from the GrainMark cohort (FFQ)	FIE-MS	Urine	• Dihydrocaffeic acid, hippuric acid, caffeic acid for coffee intake • Metabolites from other foods, including cheese, chocolate, wine, beer, and other alcoholic beverages were not reported	(32)
Coffee	Habitual coffee consumption	Frequency of coffee consumption and number of 250 mL cups each consumption (from FFQ)	Association study	564 healthy volunteers from the Hong Kong Osteoporosis Study	LC-MS (Untargeted)	Serum	• **Quinate** • **3-Hydroxypyridine sulfate** • **Trigonelline** • **AFMU** • **AAMU** • **1-Methylxanthine** • **Paraxanthine** • **3-Methyl catechol sulfate** • **1-Methylurate** • **1,7-Dimethylurate** • **3-Hydroxyhippurate**	(33)
Coffee	Coffee intake	Frequency of coffee consumption (from FFQ)	Association study	1595 women from the Nurses Health Study I and II	LC-MS/MS (Untargeted)	Plasma	• **Trigonelline** • **AAMU** • **Cinnamoylglycine** • **1,7-Dimethyluric acid** • **Caffeine** • **Phenyllactic acid** • **4-Hydroxyhippuric acid** • **Cytosine** • **7-Methylxanthine** • **L-carnitine** • **C20:4 cholesterol ester** • **C18:1 cholesterol ester**	(34)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
							• **C18:2 cholesterol ester**	
Coffee	Habitual coffee intake, boiled and filtered coffee intake	Frequency of coffee intale (including boiled and filtered) (from FFQ)	Nested case-control	421 case-control pairs and 129 at 10-year follow-up	LC-MS (Untargeted)	Plasma	Filtered coffee: • **1-Methyluric acid** • **Quinic acid** • **Theobromine** • **2-Furoylglycine** • **AAMU** • **Cyclo(leucyl-prolyl)** • **7-hydroxy-4(methoxymethyl)coumarin** Boiled coffee: • **LysoPE(20:4)** • **PE(20:4/16:0)** General: • **Caffeine** • **Paraxanthine** • **Theophylline** • **Trigonelline** • **Atractyligenin glucuronide** • **Ethyl 3-mercaptopropanoic acid** • **LysoPC(24:0)** • **LysoPE(22:4)** • **LysoPE(22:5)** • **LysoPE(24:0)** • **N-Methylpyridinium** • **Sinapic acid** • **Dihydroferulic acid 4-sulphate**	(35)
Wine	Red wine vs. no red wine	250 mL/day	4-week, randomized, controlled, parallel	33 healthy adults; 8 in control group	U(H)PLC-TOF-MS	Urine	• **(3S,5R,6S,7E,9x)-7-megastigmene-3,6,9-triol 9-glucoside** • **(E)-2-propenyl [3-(2-propenylthio)-2-propenyl] sulfate** • **1-(2,3-dihydro-1H-pyrrolizin-5-yl)-2-propen-1-one** • **2,3-Dihydroxy-3-methylvaleric acid** • **2,3-Dihydroxyvaleric acid** • **2,3-Dihydroxyvaleric acid** • **2,3-Dimethyl-3-hydroxyglutaric acid** • **2-Isopropyl-3-oxosuccinate** • **3-Carboxy-4-methyl-5-pentyl-2-furanpropanoic acid** • **3-Methoxy-4-hydroxyphenylglycol sulfate** • **3-Methylcrotonylglycine** • **4′,6′-Dihydroxy-2′-methoxyacetophenone 6′-glucoside** • **4-Chloro-3-[(2-chloro-5-nitrobenzoyl)carbamothioylamino]benzoic acid**	(36)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• 4-Hydroxy-5-(dihydroxyphenyl) valeric acid-O-sulfate • 4-Hydroxy-5-(phenyl) valeric acid-O-glucuronide • 4-Hydroxy-5-(phenyl) valeric acid-O-sulfate • 5-(1-propynyl)-5-vinyl-2,2-bithiophene • DHPV sulfate • 5,7-dihydroxy-3′,4′-dimethoxy-5′-prenylflavanone • 5-Methoxybilobetin • AFMU • Aurantricholide B • Azaspirazid • Caffeic acid • Catechol sulfate (pyrocatechol sulfate) • Coumaroyl−glucose • DHPV-O-methyl-O-sulfate • DHPV-O-glucuronide • Dibenzyl disulfide • Dihydropteridine • Ethyl 1-(ethylthio)propyl disulfide • Ethyl maltol • (*iso*)Ferulic acid sulfate • (*iso*)Ferulic acid sulfate • Galactosylglycerol • Glucosinalbin • Hesperetin-O-sulfate • Hordatine B glucoside • Hydroxytyrosol • Kanzonol I • Kanzonol R • Luteolin sulfate • L-γ-glutamyl-L-(*iso*)leucine • Methyl helianthenoate F glucoside • Methylisocitric acid • Monoglyceride citrate • O-methoxycatechol-O-sulfate • O-methoxycatechol-O-sulfate • O-ureidohomoserine • Oxovaleric acid • p-Chlorobenzenesulfonyl urea • Phenol sulfate • Phenylalanyl aspartate • Phloroglucinol • Pyrogallol sulfate • Salicylate glucuronide	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
							• **Sulfohydroxybenzoic acid** • **Tartaric acid** • **Tyrosol sulfate** • **Tyrosol sulfate** • **Valechlorin** • **Vanillic acid 4-sulfate** • **Vanillin 4-sulfate** • **Wthyl hydrogen sulfate** • **Wyeronic acid** • **α-Terpinyl cinnamate**	
Wine	Wine (administered with grapes) administered as part of a food challenge	Not reported	Acute intervention	7 healthy adults	NMR	Urine	• **2,3-Butanediol** • **2-Isopropylmalate** • **Diethylmalonate**	(37)
Wine	Red wine vs. no red wine	250 mL/day	4-week, randomized, controlled, parallel	33 healthy adults; 8 in control group	U(H)PLC-TOF (untargeted)	Feces	• **2,3-Pentanedione acid** • **2-Hydroxyglutaric acid** • **2-Methylbutyric acid** • **2-Phenethyl butyrate** • **2-Phenylethyl hexanoate** • **4-Hydroxy-5-(3-hydroxyphenyl) valeric acid** • **4-Hydroxy-5-(phenyl) valeric acid** • **Benzoic acid** • **Cholesterol sulfate** • **Deoxycholic acid** • **DHPV** • **Diethylmalonate** • **Docosahexaenoic acid methyl ester** • **Glutaric acid** • **Stercobilin** • **Urobilinogen**	(38)
					U(H)PLC-ESI-MS/MS (targeted for microbial phenolics)		• **DHBA** • **3-Hydroxyphenylacetic acid** • **3-O-Methylgallic acid** • **3-Phenylpropionate** • **4-hydroxy-5-(3,4-dihydroxyphenyl) valeric acid** • **4-Hydroxy-5-(phenyl) valeric acid** • **DHPV** • **Protocatechuic acid** • **Syringic acid** • **Vanillic acid**	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Wine	Red wine vs. no red wine	250 mL/day	4-week, randomized, controlled, parallel	33 healthy adults; 8 in control group	U(H)PLC-TOF-MS	Feces	• **2,3,-Pentanedione** • **2-Hydroxyglutaric acid** • **2-Methylbutyric acid** • **2-Phenethyl butyrate** • **2-Phenylethyl hexanoate** • **4-Hydroxy-5-(3-hydroxyphenyl) valeric acid** • **4-Hydroxy-5-(phenyl) valeric acid** • **Benzoic acid** • **Cholesterol sulfate** • **Deoxycholic acid** • **DHPV** • **Diethylmalonate** • **Docosahexaenoic acid methyl ester**	(39)
Wine	Red wine, dealcoholized vs. red wine, alcoholized vs. gin (comparator)	272 mL/day (wines); 100 mL/day (gin)	28-day, randomized, controlled, crossover	61 healthy men with high CV risk	NMR	Urine	• **3-Methyl-2-oxovalerate** • **4-Hydroxyphenylacetate** • **Ethanol** • **Hippurate** • **Mannitol** • **Tartrate** • **Trigonelline** • 1-Methylnicotinamide • 2-Hydroxyisobutyrate • 3-Hydroxyisobutyrate • 3-Hydroxymandelate • Acetate • Acetoacetate • Acetone • Acetylcarnitine • Alanine • Betaine • Carnitine • cis-Aconitate • Citrate • Creatine • Creatinine • Dimethylamine • Formate • Fucose • Glucose • Glycine • Glycylproline • Histidine • Indole-3-acetate	(40)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
							• Lactate • Leucine • Lysine • Malonate • Methylsuccinate • N-Methylhistidine • N-N • N-Phenylacetylglycine • Succinate • Taurine • Threonine • Trimethylamine • Trimethylamine-N-oxide • Tyrosine • Urea • Valine	
Wine	Red wine, dealcoholized; Red wine, alcoholized; Gin (comparator)	272 mL/day (wines); 100 mL/day (gin)	28-day, randomized, controlled, crossover	56 adults/elderly with high CV risk	NMR	Urine	• **2,3-Butanediol** • **2-methyl-2-oxovalerate** • **4-Hydroxyphenylacetate** • **Ethanol** • **Ethyl glucuronide** • **Hippurate** • **Mannitol** • **Tartrate**	(41)
	Wine	0 mL/day (non-consumer); <180 mL/day (intermittent); ≥180 mL/day (consumer)	5-year, multi-centre, randomized, controlled, parallel, single-blind (baseline assessment)	91 adults/elderly with high CV risk			• **2,3-Butanediol** • **2-methyl-2-oxovalerate** • **Ethanol** • **Ethyl glucuronide** • **Mannitol** • **Tartrate**	
Wine	Red wine, dealcoholized vs. no wine	272 mL/day	28-day, randomized, controlled, crossover	57 adults/elderly with high CV risk	NMR	Urine	• **3-Hydroxyphenylacetic acid** • **4-Hydroxyphenylacetate** • **Betaine** • **Dimethylamine** • **Fucose** • **Glucose** • **Lactate** • **Mannitol** • **Methanol** • **Tartrate** • **Threonine**	(42)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cheese	Cheese (hard, yellow Samsø)	143 g/day	6-week, randomized, controlled, crossover	23 healthy adults	U(H)PLC-QTOF-MS	Urine	• 4-Hydroxyphenylacetate • Indoxyl sulfate • Isobutyrylglycine • Isovalerylglutamic acid • Isovalerylglycine • Triglylglycine • Tyramine sulfate • Xanthurenic acid	(43)
Cheese	Cheese (Gruyere)	100 g + 500 mL water	Acute, randomized, controlled, crossover	11 healthy adults	GC-MS	Urine	• **3-Phenyllactic acid** • **4-Methylcatechol** • **Alanine** • **Lactic acid** • **Pyroglutamic acid**	(44)
					NMR		• **Alanine** • **Proline** • **Pyroglutamic acid**	
Cheese	Cheese (semihard, cow)	1.859 g/day	2-week, randomized, controlled, crossover	15 healthy men	NMR	Feces	• **Acetate** • **Butyrate** • **Malonate** • **Propionate**	(45)
						Urine	• **Hippurate** • **Proline betaine** • **Tyrosine** • **Urea**	
Cheese	Cheese vs. milk vs. soy beverage	100 g (cheese), 600 mL (milk or soy beverage)	Acute, randomized, controlled, crossover	11 healthy volunteers	HS-GC-MS (Untargeted)	Plasma, urine (volatile)	• **Heptan-2-one (plasma)** • **Undecan-2-one (plasma)** • **Heptan-4-one (urine)**	(46)
Cheese	Cheese vs. milk vs. soy beverage	100 g (cheese); 600 mL (milk or soy beverage)	Acute, randomized, controlled, crossover	11 healthy volunteers	LC-MS (Untargeted)	Plasma, urine	• **Aminoadipic acid** • **Citrulline** • **Valyl-threonine** • **Phenylalanyl-proline** • **Indolelactic acid** • **Proline**	(47)
Cheese	Cheese (Gruyere) vs. baseline	100 g + 500 mL water	Acute, randomized, controlled, crossover	11 healthy adults	GC-MS	Serum	• **Methionine** • **Proline** • **Leucine** • **Glutamic acid** • **3-Phenyllactic acid** • **Pentadecanoic acid (C15)** • **Heptadecanoic acid (C17)** • Lactose	(48)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
							• Galacitol • Galactono-1,5-lactone • Dodecanoic acid • Linoleic acid • Gamma-tocopherol • Maltol • Sucrose • Guaiacol • Catechol	
					NMR		• **Methionine** • **Tyrosine** • **Valine+isoleucine** • **3-Hydroxyisobutyrate** • Lipid=CH-CH2-CH=	
Fermented milk	Probiotic fermented milk	0.4 L/day	8-week, randomized, controlled, parallel	31 IBS patients	NMR	Serum	• **3-Hydroxybutyrate** • **D-Lactate** • **L-lactate**	(49)
Fermented milk	Probiotic fermented milk	0.4 L/day	8-week, randomized, controlled, parallel	31 IBS patients	GC-MS	Serum	• **Aspartic acid** • **Creatine/creatinine** • **Glutamine** • **Lactate** • **Proline**	(50)
Yoghurt	Yoghurt vs. baseline	800 g	Acute, randomized, controlled, double-blind, crossover	14 healthy men	LC-MS	Serum	• **Phenylalanine** • **Threonine** • **Lysine** • **Proline** • **Asparagine** • **Tyrosine** • **Tryptophan** • **Citrulline** • **Indole-3-lactic acid** • **Indole-3-acetaldehyde**	(51)
			2-week, randomized, controlled, double-blind, crossover				• **Proline** • **Lysine** • **Threonine** • **Citrulline** • **Indole-3-lactic acid** • **Indole-3-acetaldehyde**	
Beer, coffee, wine	Beer	Habitual intake (dose not reported);	Case-control	125 patients with colon adenoma; 128 controls	U(H)PLC-MS/MS and GC-MS	Urine	• **Glycerol 3-phosphate** • **Homovanillate sulfate**	(52)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
		assessed by FFQ						
	Coffee, caffeinated	Habitual intake (dose not reported); assessed by FFQ				Serum	• **1,3,7-Trimethylurate** • **1,3-Dimethylurate** • **1,7-Dimethylurate** • **1-Methylxanthine** • **Caffeine** • **Catechol sulfate** • **N-(2-furoyl)glycine** • **Quinate** • **Theophylline** • **Trigonelline**	
	Coffee, caffeinated	Habitual intake (dose not reported); assessed by FFQ				Urine	• **1,3,7-Trimethylurate** • **1,3-Dimethylurate** • **1,7-Dimethylurate** • **1-Methylurate** • **1-Methylxanthine** • **AAMU** • **AFMU** • **Caffeine** • **Catechol sulfate** • **Hippurate** • **Homovanillate sulfate** • **N-(2-furoyl)glycine** • **Nicotinate** • **Pseudouridine** • **Quinate** • **Theophylline** • **Trigonelline**	
	Coffee, decaffeinated	Habitual intake (dose not reported); assessed by FFQ				Serum	• **1,7-Dimethylurate** • **3-methoxytyrosine**	
	Wine	Habitual intake (dose not reported); assessed by FFQ				Urine	• **2,3-Dihydroxyisovalerate** • **2-Isopropylmalate** • **Nicotine**	
Beer	Strong lager vs. regular lager vs. light/alcohol-free beer vs.	330 mL	3-day, randomized, controlled,	37 healthy adults	U(H)PLC-QTOF-MS	Plasma	• Hydroxy alloiso-cohumulone, cohumulinone • 2-ethyl malate • Cysteine conjugate of NO2 or CH2O2 adducted *iso*-ad/humulone	(53)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	control soft drink		single-blind, crossover				• Cysteine conjugate of NO2 or CH2O2 adducted *iso*-cohumulone • Dihydroxylated *iso*-ad/humulone • Dihydroxylated *iso*-ad/humulone II • Hordenine • Hydroxy alloiso-ad/humulones, humulinone • Iso/leucine • Iso-cohumulone • Iso-n/ad-humulone • Iso-tricycload/humene • Iso-tricyclohumene • Maltose • N-methyl tyramine sulfate • NO or CH2O adduct of *iso*-ad/humulone • NO or CH2O adduct of *iso*-cohumulone • NO2 or CH2O2 adduct of *iso*-cohumulone • NO2 or CH2O2 conjugate adduct of *iso*-ad/humulone • Pyroglutamyl proline • Tetra-cycload/humol • Tetra-cyclocohumol, tricyclohumol • Tricycload/humol, double hydroxylated *iso*-cohumulone • Tyrosine	
Beer	Beer, alcoholic	660 mL/day	4-week, randomized, controlled, crossover	33 men with high CV risk	HPLC-ESI-MS/MS (targeted plasma acylcarnitines)	Plasma	• Acylcarnitines	(54)
					LC-MS (untargeted)		• **1,2,3,4-Tetrahydro-1-methyl-beta-carboline-3-carboxylic acid** • **2,3-Dihydroxy-3-methylvaleric acid** • **2-Phenylethanol glucuronide** • **4-Guanidinobutanoic acid** • **Cohumulone** • **Ethyl glucuronide** • **Ethyl sulfate** • **Humulinone** • **Hydroxyadipic acid** • **Oxyhumulinic acid**	
	Beer, non-alcoholic	660 mL/day			HPLC-ESI-MS/MS (targeted plasma acylcarnitines)		• Acylcarnitines	

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods

Fermented Food	Intervention	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
					LC-MS (untargeted)		• **2,3-dihydroxy-3-methylvaleric acid** • **4-Guanidinobutanoic acid** • **Cohumulone** • **Humulinone** • **Hydroxyadipic acid** • **Oxyhumulinic acid**	
Beer	Habitual dietary intake	Frequency of intake for 137 foods, including beer (from FFQ)	Association study	491 volunteers from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial	HILIC-QQQ-MRM (Untargeted)	Serum	• **Beer: C24:0 sphingomyelin**	(55)
Rice beer	Rice beer drinkers vs. non-drinkers In Ahom and Bodo ethnic groups	Drinkers or non-drinkers of rice beer (from questionnaire)	Cross-sectional	134 healthy volunteers	GC-MS (Untargeted)	Feces	• Propanoic, butyric, cis-vaccenic acids was higher in Ahom non-drinkers vs. drinkers • Butyric acid, rhamnose, arabinose, glycine, hydroxycinnamic acid, indole, formic acid, ursodeoxycholic acid, acetic acid, and benzoic acid was higher in Bodo non-drinkers vs. drinkers (250 metabolites were detected, but only 40 metabolites from microbial origin were considered)	(56)
Post-fermented tea	Tea, pu-erh	200 mL/day	2-week, randomized, crossover	20 healthy adults	U(H)PLC-QTOF-MS	Urine	• **1,3-Dimethylurate** • **1,7-Methyluric acid** • **1-Methyluric acid** • **1-Methylxanthine** • **2-Hydroxybenzoic acid** • **2-Methoxyphenol** • **3,5-Hydroxybenzoic acid** • **3-Hydroxyphenylacetic acid** • **4-Aminobutanoic acid** • **4-Aminobutanoic acid** • **4-Hydroxy-3-methoxyphenylacetic acid** • **Aminomalonic acid** • **Caffeine** • **Epigallocatechin** • **Hippurate** • **Nicotinic acid** • **Ornithine** • **Paraxanthine** • **Phenol** • **Theobromine** • **Theophylline** • **Valine**	(57)

Table S3. Summary of untargeted studies presenting candidate FIBs for fermented foods								
Fermented Food	**Intervention**	**Dose**	**Study Design**	***n***	**Analytical Method**	**Biosample**	**Candidate FIBs[a]**	**Reference**
General fermented foods	Fermented food intake frequency (never, <1X/week, 1-2X/week, 3-5X/week, daily)	Habitual diet and activites; dose not reported	Cross-sectional	7 healthy adults who ferment their own foods	U(H)PLC-MS	Multiple (biofilm, fermented food, forehead, indoor surface, hands, mouth, stool)	• **Avobenzone (skin)** • **Bacteriopheophytin (kimchi)** • **Cholesterol and derivatives (skin)** • **Gingerol (foods, indoor surfaces)** • **Pheophytin A (foods of vegetable origin)** • **Piperine (food, stool, indoor surfaces, skin)** • **Plant flavonoids, lipids, plant sterols** • **Polanrazine B (food, stool)** • **Procyanidin B2 (biofilm, food, indoor surface, skin, stool)** • **Pyropheophytin (kimchi)**	(58)

AAMU, 5-Acetylamino-6-amino-3-methyluracil; AFMU, 5-Acetylamino-6-formylamino-3-methyluracil; AMMU, 6-amino-5[N-methylformylamino]-1-methyluracil; AR, alkenylresorcinol; DCER, dihydroceramides; DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-1-propanoic acid); DHPPTA, 5-(3,5-dihydroxyphenyl) pentanoic acid; DHPV, 5-(3,4-dihydroxyphenyl)-γ-valerolactone; ESI, electrospray ionization; FA, fatty acid; FFQ, food frequency questionnaire; FIB = food intake biomarker; FIE, flow injection electrospray; GC, gas chromatography; HBOA, 2-Hydroxy-1,4-benzoxazin-3-one; HHPAA, 2-Hydroxy-N-(2-hydroxyphenyl) acetamide; HILIC, hydrophilic interaction liquid chromatography; HPAA, N-(2-hydroxyphenyl) acetamide; HPLC, high-performance liquid chromatography; HS, headspace; IMS, ion mobility spectrometry; LC, liquid chromatography; LCER, lactosylceramides; MHPV, 3′-methoxy-4′-hydroxyphenylvalerolactone; MS, mass spectrometry; MRM, multiple reaction monitoring; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; QQQ, triple quadrupole; QTOF, quadrupole time-of-flight; SPME, solid phase microextraction; TAG, triacylglycerol; TOF, time-of-flight; U(H)PLC, ultra-high performance liquid chromatography.

[a] Candidate FIBs that are significantly increased compared to control or baseline in each study are bolded. Candidate FIBs that are detected in the biosample, but not statistically significant, are not bolded.

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Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Beer	Beer vs. Non-alcoholic control drink	330 mL	Acute, randomized, controlled, crossover	19 healthy volunteers (*n* = 4 urine, *n* = 3 plasma, *n* = 1 serum)	U(H)PLC-MS/MS (Targeted)	Serum, plasma, urine	• L-Tartaric acid • Ethyl sulfate • Ethyl-beta-D-gulcuronide • Indoxyl sulfate • Cresol sulfate • Resveratrol • Estrone sulfate • Dihydroepiandrosterone sulfate • (Isoxanthohumol)* • (Isocohumulone)* • (3-nitrotyrosine)* • (Indole-3-lactic acid)* • (Cortisol)* • (Cortisol sulfate)* (* Detected but not validated)	(1)
Beer	Amstel beer (RIAA) vs. Hahn Premium Light beer (TIAA) vs. Coopers Clear beer (HIAA)	470-770 mL (Amstel), 850-1500 mL (Hahn), 500-850 mL (Coopers Clear)	Acute intervention	5 healthy volunteers	U(H)PLC-MS/MS (Targeted)	Urine	• Iso-alpha-acids (isohumulones) • Rho-*iso*-alpha-acids (RIAA) • Tetrahydro-*iso*-alpha-acids (TIAA) • Hexahydro-*iso*-alpha-acids (HIAA)	(2)
Beer	Beer	330 mL	Acute intervention	10 healthy volunteers	LC-ESI-MS/MS (Targeted)	Urine	• Isoxanthohumol • Xanthohumol • 8-prenylnaringenin	(3)
Beer	Beer + 300 g allopurinol (anti-hyperuricemic agent) vs. Beer alone vs. Allopurinol alone	10 mL/kg body weight	Acute crossover	5 healthy volunteers	Not reported (Targeted)	Plasma, urine	• Hypoxanthine • Xanthine • Uric acid	(4)
Beer	Volt-Damm beer	330 mL (women); 660 mL (men)	Acute intervention	7 healthy volunteers	Melatonin ELISA and HPLC (Targeted)	Serum	• Melatonin	(5)
Beer	Beer vs. Vodka and carbonated water	1 L (standardized to 48 g alcohol content)	Acute crossover	7 healthy volunteers	GC-MS/MS (Targeted)	Urine, serum	• Mevalonic acid	(6)
Beer	Little Creatures Pale Ale (high-hopped) vs. Erdinger	613 to 802 mL	Acute crossover	5 healthy men	U(H)PLC-MS/MS (Targeted)	Whole blood, urine	• Iso-alpha-acids (isohumulones)	(7)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	Weissbier (low-hopped)							
Beer	1 Beer, 2 Beers, 3 Beers (male); 1 Beer, 1.5 Beers, 2 Beers (female)	330 mL, 660 mL, 990 mL (males); 330 mL, 495 mL, 660 mL (females)	Acute dose-response, randomized, crossover	41 healthy volunteers	LC-MS/MS (Targeted)	Urine	• Isoxanthohumol	(8)
	Beer vs. Gin vs. non-alcoholic beer	660 mL (beer); 92 mL (gin) (standardized to 30 g ethanol/day); 990 mL non-alcoholic beer (equivalent amount of polyphenols)	4-week randomized, open, controlled, crossover	33 males with high CV risk				
	Beer	No beer drinkers vs. intermittent/daily beer drinkers (22 to 825 mL/day) (from FFQ)	Parallel-group, multicenter, controlled, randomized 5-year clinical trial	46 volunteers from PREDIMED cohort				
Beer	Non-alcoholic beer	2.5 L	Acute intervention	4 healthy volunteers	LC-MS/MS (Targeted)	Urine	• Ethyl glucuronide • Ethyl sulfate	(9)
Beer	Low-alcohol beer	4 L over 4 hours	Acute intervention	5 healthy men	HPLC-PDA (Targeted)	Urine	• Ferulic acid (total, free and glucuronidated) • 4-Dydroxy-3-methoxy-cinnamic acid	(10)
Beer	Beer	0.5 g/kg body weight (594 to 986 mL)	Acute intervention	6 healthy volunteers	LC-MS (Targeted)	Urine	• Ethanol • Ethyl glucuronide	(11)
Beer	Light beer	330 mL	Acute intervention	8 healthy volunteers	Enzymatic method (Targeted)	Capillary blood	• Ethanol	(12)
	Beer on empty stomach	660 mL	Acute intervention	9 healthy men				
	Beer with meal	660 mL	Acute intervention	9 healthy men				

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Beer	Low-alcohol beer (0.5%) vs. low-alcohol beer (0.9%)	3 L	Acute, randomized, crossover	20 healthy men	Alcohol dehydrogenase method (Targeted)	Blood	• Ethanol	(13)
Beer	Beer vs. whisky vs. white wine vs. dry sherry	~614 mL (beer); 240 mL (beer); 157 mL (sherry); 66.6 mL (whisky); standardised to 0.3 g/kg body weight ethanol	Acute, randomized, crossover	11 healthy men	Breathalyser (Targeted)	Blood	• Ethanol	(14)
Beer	Non-drinkers vs. drinkers after a drinking party	0 (non-drinkers); not reported (drinking party)	Cross-sectional	40 healthy men (non-drinkers); 13 healthy men (drinking party)	GC-MS (Targeted)	Urine	• 1-Methyl-1,2,3,4-tetrahydro-beta-carboline • 1,2,3,4-Tetrahydro-beta-carboline • Tryptamine (after administration of dideuterated L-tryptophan)	(15)
	Beer vs. whisky	500 mL (beer); 68 mL (whisky)	Acute intervention	4 healthy men				
Beer	Beer	16 mL/kg body weight	Acute intervention	4 healthy men (3 flushers and 1 non-flusher)	HS-GC (Targeted)	Blood, urine	• Ethanol • Acetaldehyde • Acetate	(16)
Beer	Beer	16 mL/kg body weight (non-flushers); 8 mL/kg (flushers)	Acute intervention	4 healthy men	HS-GC-FID (Targeted)	Urine	• Free and bound ethanol • Free and bound acetaldehyde • Free and bound acetate • Free and bound acetone • Free and bound methanol	(17)
Beer	Beer vs. rum vs. carbonate mixed rum	275 mL (beer); 40 mL (rum); standardised to 12 g ethanol	Acute parallel	30 healthy volunteers	TDx Abbott analyzer (Targeted)	Plasma	• Ethanol	(18)
Beer	Tusker or Pilsner beer	8-15 beers (500 mL each) for me; 8-12 beers for women	Acute intervention	17 volunteers	GC (detector not specified) (Targeted)	Urine, blood	• Alcohol	(19)
Beer	Beer	762 to 1000 mL	Acute intervention	4 healthy volunteers	HS-GC-EI-MS (ethanol), U(H)PLC-ESI-MS/MS (hordenine) (Targeted)	Plasma	• Ethanol • Hordenine (N,N-dimethyltyramine) and glucuronidated and sulfated conjugates	(20)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Wine, beer	Consumption of 10 alcoholic beverages (light/medium/full strength beer; red/ white/sparkling wine; wine cooler, spirits/liqueurs, spirit-based mixed drinks, sherry/port, and other)	Frequency of intake (never or <1/month, 1–3 times/month, once/week, 2–4 times/week, 5–6 times/week, once/day, 2–3 times/day, 4–5 times/day, >6 times/day) for 10 alcoholic beverages (from FFQ)	Cross-sectional	1785 healthy volunteers from the Childhood Determinants of Adult Health study	NMR (Targeted)	Serum	• Weak positive associations for fatty acids: total fatty acids, saturated fatty acids, MUFA, PUFA, omega-6 PUFA, linoleic acid, omega-3 PUFA, DHA (total alcohol, wine beer) • Weak positive associations for low-molecular weight metabolites: Alanine (total, wine, beer), glutamine (wine), tyrosine (total, wine, beer), glucose (total, wine, beer), pyruvate (beer), glycerol (beer), acetoacetate (beer), beta-hydroxybutyrate (beer), albumin (beer), acetate (beer)	(21)
Wine, beer	Red wine vs. beer vs. Dutch gin vs. water	4 glasses; standardised to 40 g/day alcohol	3-week, randomized, controlled, crossover	12 healthy men	HPLC-FLD/UV (Targeted)	Plasma	• Alpha-tocopherol • Gamma-tocopherol • Lutein • Beta-cryptoxanthin • Lycopene • Alpha-carotene	(22)
Wine, beer	Red wine vs. Lager beer vs. Stout (alcoholic) vs. Stout (non-alcoholic) vs. Water with alcohol	1 drink: 341 mL 3 drinks: 3 X 155 mL (wine); 3 X 341 mL (lager beer); 3 X 341 mL (stout), 3 X 341 mL (water)	Acute intervention	12 healthy volunteers (1 drink); 8 healthy volunteers (3 drinks)	Sigma Alcohol kit, ELISA reader (Targeted)	Plasma	• Ethanol	(23)
Wine, beer	Alcohol intake (wine, beer and spirits)	Quartiles of alcohol intake as % total energy intake: Q1:0, Q2: 0.6, Q3: 2.3, Q4: 5.8 (from FFQ)	Association study	1457 healthy volunteers from the IMMIDIET cohort	GC-FID (Targeted)	Plasma	• EPA (women and men) • DHA (women)	(24)
Wine, beer	Beer, white wine, vodka/tonic	All beverages give 0.5 g ethanol/kg body weight (5.1% v/v beer, 12.5% v/v chardonnay, 20% v/v vodka and tonic)	Acute crossover	15 healthy men	HS-GC (Targeted)	Whole blood	• Ethanol	(25)
Wine, beer	Pilsen-type beer, Cabernet Sauvignon red	All beverages give 0.5 g ethanol/kg body weight	Acute crossover	20 healthy volunteers	COBAS INTEGRA Ethanol Kit (Targeted)	Plasma	• Ethanol	(26)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	wine, Scotch whisky, cachaca							
Wine, beer	Swedish vodka and tonic (Absolut), French wine (La Garonne), Swedish export beer (Pripps Export)	All beverages give 1.0 mL ethanol/kg body weight	Acute intervention	6 healthy men	Not reported	Blood	• Ethanol	(27)
Wine, beer	White wine or beer	0.1 or 0.2 L (white wine); 0.33 L or 0.66 L (beer)	Acute intervention	12 healthy volunteers	LC-MS/MS (Targeted)	Urine	• Ethyl glucuronide • Ethyl sulfate	(28)
Wine, beer	Choice of white wine or export beer	50 g or 80 g ethanol	Acute intervention	20 healthy volunteers ('social drinkers')	HS-GC, GC-MS, HPLC (Targeted)	Urine	• Ethanol • Methanol • 5-Hydroxytryptophol • 5-Hydroxyindol-3-ylacetic acid	(29)
Wine	Dealcoholized red wine	272 mL/day	4-week, randomized, controlled intervention	36 elderly men	U(H)PLC-MS/MS (Targeted 67 metabolites)	Urine	• (Epi)catechin glucuronides • (Epi)catechin sulfates • DHPV glucuronides • DHPV sulfates • Ethylgallate glucuronides • Ethylgallate sulfate • Methyl(epi)catechin glucuronides • Methyl(epi)catechin sulfates • Methylgallic sulfate • MHPV glucuronide • MHPV sulfates • Hydroxybenzoic acids (especially gallic acid) • Hydroxyphenylacetic acids • Hydroxycinnamic acids • Hydroxyphenylpropanoic acids • Glycinates (vanilloylglycine) • Hydroxyphenylvalerolactones • Enterolactone • Pyrogallol	(30)
Wine	Sparkling wine	0.1 to 0.2 L (9 or 18 g ethanol); 1 volunteer drank	Acute intervention	9 healthy volunteers	LC-ESI-MS/MS (Targeted)	Urine	• Ethyl sulfate • Ethyl glucuronide	(31)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
		0.54 L (49 g ethanol)						
Wine	Merlot red wine	250 mL	Acute intervention	11 healthy men	HPLC-MS/MS (Targeted)	Urine	• Resveratrol (trans-resveratrol) and metabolites (trans-piceid, taxifolin, hexestrol, trans- and cis-resveratrol glucuronides and sulfates)	(32)
Wine	Red wine vs. dealcoholized red wine	272 mL/day	28-day, randomized, controlled, crossover	73 volunteers with high cardiovascular risk	U(H)PLC-MS/MS (Targeted)	Urine	• Resveratrol (trans- and cis-resveratrol) and metabolites (trans-piceid, piceid glucurondies and sulfates, trans- and cis-resveratrol glucuronides and sulfates, dihydroresveratrol and its glucuronides and sulfates)	(33)
Wine	Red wine vs. dealcoholized red wine	272 mL/day	4-week, randomized, controlled, crossover	36 men with high cardiovascular risk	U(H)PLC-MS/MS (Targeted 70 phenolic metabolites)	Plasma, urine	Plasma: • 3-Hydroxyphenylacetic acid • DHPV and glucuronides • Gallic acid • Methyl(epi)catechin glucuronides • Methylgallic acid • Methylgallic sulfate • p-Coumaric acid • (Epi)catechin glucuronides • 3-Hydroxyphenylacetic acid Urine: • (Epi)catechin glucuronides • (Epi)catechin sulfates • 2,4-Dihydroxybenzoic acid • 2,5-Dihydroxybenzoic acid • 2,6-Dihydroxybenzoic acid • 3-Hydroxyphenylacetic acid • DHPV and glucuronides and sulfates • Ethylgallate and glucuronides and sulfates • Gallic acid • Methyl(epi)catechin glucuronides • Methyl(epi)catechin sulfates • Methylgallic acid • Methylgallic sulfate • p-Coumaric acid • Pyrogallol • Resveratrol • Syringic acid • Vanilloylglycine • 3-(3-hydroxyphenyl) propionate	(34)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• DHBA • 3-Hydroxybenzoic acid • 3-Hydroxyphenylacetic acid • 4-Hydroxybenzoic acid • Caffeic acid • Enterolactone • Ferulic acid • MHPV glucuronides	
Wine	White wine vs. red wine	300 mL/day	15-day, randomized, controlled, parallel	20 healthy volunteers	HPLC electrochemical method (Targeted)	Plasma	• Resveratrol	(35)
Wine	Wine vs. grape juice vs. grape tablets enriched with trans-resveratrol (also compared to separate study with olive oil)	250 mL/day (wine); 1L/day (grape juice); 1 mg resveratrol/day (tablets)	4-day, randomized, controlled, crossover	12 healthy volunteers	GC-MS (Targeted)	Plasma	• Hydroxytyrosol and its metabolite, homovanillic alcohol	(36)
Wine	Red wine vs. no wine	250 mL/day	4-week, randomized, controlled, parallel	41 healthy volunteers	U(H)PLC-ESI-MS/MS (Targeted microbial-derived phenolics)	Feces	• 3,5-Dihydroxybenzoic acid • 3-Hydroxyphenylacetic acid • 3-Phenylpropionate • 4-hydroxy-5-(3,4-dihydroxyphenyl) valeric acid • 4-Hydroxy-5-(phenyl) valeric acid • 4-O-methylgallic acid • DHPV • Protocatechuic acid • Syringic acid • Vanillic acid	(37)
Wine	Wine, grapes, peanuts, and red wine intake (along with total resveratrol intakes)	Continuous intake of resveratrol, resveratrol 3-O-glucoside, resveratrol aglycone, wine, grapes, peanuts, and red wine (from 24-h recalls and dietary questionnaire)	Association study	475 volunteers from the EPIC cohort	U(H)PLC-MS/MS (Targeted)	Urine	• Resveratrol	(38)

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Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Wine	White wine (and crackers)	250 mL	Acute intervention	10 healthy volunteers	HPLC-ECD (Targeted)	Plasma	• Caffeic acid • Ferulic acid • P-coumaric acid	(39)
Wine	White wine	Volume to achieve ~0.5 g/kg body weight ethanol	Acute intervention	13 healthy volunteers	LC-MS/MS (Targeted)	Serum, urine	• Ethyl glucuronide • Ethyl sulfate	(40)
Wine	Extra virgin olive oil, red wine, extra virgin olive oil + red wine	25 mL (EVOO), 150 mL (wine)	Acute, randomized, crossover	12 healthy volunteers	HPLC-MS/MS (Targeted)	Urine	• Resveratrol (cis, trans, dihydro) • Tyrosol (free, sulfate, and glucoronide) • Hydroxytyrosol (free, sulfate, and glucoronide) • Ethyl glucuronide (wine)	(41)
Wine	Red wine vs. dealcoholized red wine	200 mL	Acute crossover	10 healthy volunteers	HPLC-Coulochem II detector (Targeted)	Plasma	• Caffeic acid	(42)
Wine	Test meal (Milanese beef cutlet and chips) with red wine	300 mL	Acute intervention	10 healthy men	HPLC-UV and HPLC-PDA-MS (Targeted)	Serum	• Trans-resveratrol (and 3- and 4' glucuronides)	(43)
	Red wine	600 mL	Acute intervention	5 healthy volunteers	HPLC-UV-DAD and LC-MS/MS (Targeted)		• Trans-resveratrol (and 3- and 4' glucuronides)	
	'Fat meal' with red wine vs. 'lean meal' with red wine	600 mL	Acute parallel	10 healthy volunteers			• Trans-resveratrol (and 3- and 4' glucuronides)	
Wine	Red wine vs. dealcoholized red wine	272 mL/day	20-day, randomized, controlled, crossover	8 healthy volunteers	U(H)PLC-ESI-MS/MS (Targeted for microbial phenolics)	Feces	• 3,5-Dihydroxybenzoic acid • 3-O-Methylgallic acid • 4-Hydroxy-5-(phenyl) valeric acid • p-Coumaric acid • Phenylpropionic acid • Protocatechuic acid • Syringic acid • Vanillic acid	(44)
Wine	Red wine vs. dealcoholized red wine	120 mL	Acute crossover	9 healthy volunteers	GC-MS (Targeted)	Urine	• Catechin (free) and metabolites (catechin sulfate, glucuronide, glucuronide-sulfate) • 3'-methylcatechin and glucuronides and sulfates	(45)

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Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Wine	Red wine vs. alcohol abstinent	150 mL/day (females); 300 mL/day (males)	3-month, randomized, parallel	44 healthy volunteers	LC-MS/MS (Targeted)	Whole blood	• Phosphatidylethanol • Carbohydrate-deficient transferrin	(46)
Wine	Red wine, ethanol, water	155 mL (wine, first dose), ethanol equivalent to acheive BAC of 40 mg/mL 310 mL (wine, second dose)	Acute, 2-dose, randomized, single-blind, crossover	13 healthy volunteers	GC (detector not specified) (Targeted)	Plasma	• Resveratrol • Catechin	(47)
Wine	Red wine (at 3 different doses)	100 mL, 200 mL, or 300 mL	Acute, randomized, crossover	21 healthy men	LC-ESI-MS/MS (Targeted)	Urine	• Tartaric acid	(48)
Wine	Mediterranean diet vs. occidental diet, with or without red wine	240 mL/day	3-month, randomized, parallel (1-month wine intake during second month)	42 healthy men	HPLC-electrochemical detection (beta-carotene), spectrophotometry (L-ascorbic acid), Folin-Ciocalteu method (polyphenols) (Targeted)	Plasma, urine (polyphenols)	• Vitamin C • Beta-carotene • Polyphenols (gallic acid equivalents)	(49)
Wine	Wine	Non-wine consumers, intermittent wine consumers, daily wine consumers (from FFQ)	Prospective cohort	1000 volunteers with high CV risk from the PREDIMED cohort	LC-MS/MS (Targeted)	Urine	• Resveratrol metabolites (trans- and cis-resveratrol glucuronides and sulfates)	(50)
Wine	Mencia red wine	1 glass/serving per day (volume not specified)	3-day intervention	25 healthy volunteers who are occasional wine drinkers	HPLC-MS/MS (Targeted)	Plasma	• Resveratrol (trans)	(51)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Wine	Sparking wine vs. gin	300 mL/day (wine); 100 mL (gin), standardized to 30 g ethanol/day	28-day, randomized, controlled, crossover	10 healthy men	LC-MS/MS (Targeted)	Urine	• Total resveratrol metabolites (cis- and trans- resveratrol glucuronides)	(52)
	White wine vs. red wine	200 mL/day, standardized to 20 g ethanol/day	28-day, randomized, controlled, crossover	10 healthy women				
	Wine consumed at 3 different levels	Daily consumption, intermittent consumption, no consumption (from FFQ)	Prospective cohort	52 volunteers from the PREDIMED cohort				
Wine	Red wine vs. dealcholized red wine	120 mL	Acute, randomized, crossover	9 healthy volunteers	GC-MS (Targeted)	Plasma	• Catechin and 3'-O-methylcatechin (and glucuronide and sulfate conjugates)	(53)
Wine	Delacoholized red wine reconstituted with water vs dealcoholized red wine reconstituted with water and ethanol	120 mL	Acute, randomized, crossover	9 healthy volunteers	GC-MS (Targeted)	Plasma	• Total (+)-Catechin	(54)
Wine	Alcohol consumption, wine consumption	3 categories for total alcohol consumption, wine consumption (from FFQ)	Prospective cohort	1045 volunteers with high CV risk from the PREDIMED cohort	GC-MS (hydroxytyrosol); enzyme immunochemical method (ethyl glucuronide) (Targeted)	Urine	• Hydroxytyrosol • Ethyl glucuronide	(55)
Wine	Red wine vs. grape juice vs. red wine extract tablets	250 mL (wine); 1 L (juice); 10 tablets; standardized to 14 ug/kg resveratrol	Acute, randomized, controlled, crossover	11 healthy men	GC-MS (Targeted)	Plasma, urine	• Resveratrol (trans, cis), dihydroresveratrol	(56)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Wine	Red wine	200 mL	Acute intervention	5 healthy men	LC-ESI-MS/MS (Targeted)	Urine	• Tartaric acid • Malic acid • Succinic acid	(57)
Wine	Alcohol consumption	Quartiles of alcohol consumption, continous intake of wine (from FFQ)	Association study	1000 volunteers with high CV risk from the PREDIMED cohort	LC-MS/MS (Targeted)	Urine	• Total resveratrol metabolites (trans- and cis-resveratrol glucuronides and sulfates)	(58)
Wine	Merlot red wine	250 mL	Acute intervention	11 healthy men	LC-ESI-MS/MS (Targeted)	Plasma LDL	• Trans-resveratrol and metabolites (3-O-glucuronide, cis-3-O-glucuronide, cis-3-O-glucoside)	(59)
Wine	Diet without vegetable, fruit, and wine vs. diet with vegetable and fruit vs. diet with wine and no vegetable or fruit vs. diet with wine, vegetable, and fruit	Grouped intakes of fruit, vegetabe, and wine intake (from 24-h recall)	Cross-sectional	180 healthy free-living volunteers	HPLC-FLD (Targeted)	Plasma	• (+)-Catechin	(60)
Wine	Red wine vs. no red wine	375 mL/day	2-week, randomized, controlled, parallel	20 healthy free-living volunteers	HPLC-MS/MS (Targeted)	Plasma	• Total phenolics • (+)-Catechin glucuronide • (-)-Epicatechin glucuronide • Methyl catechin glucuronide • Methyl epicatethin glucuronide	(61)
Wine	Red wine	100, 200, 300 mL	Acute, dose-response intervention	5 healthy men	HPLC-Coulochem II detector (Targeted)	Plasma	• Caffeic acid	(62)
Wine	Table red wine vs. Port red wine	250 mL (table red wine); 150 mL (Port red wine)	Acute crossover	4 healthy men	HPLC-DAD, HPLC-MS (Targeted)	Plasma, urine	• Total plasma anthocyanins and metabolites • Delphinidin-3-O-glucoside • Petunindin-3-O-glucoside • Peonidin-3-O-glucoside • Delphinidin-glucuronide • Peonidin-glucuronide • Malvidin-3-O-beta-glucuronide • Malvidin-3-O-beta-glucoside	(63)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Wine	Oak-aged red wine vs. strawberries vs red raspberries vs. walnuts (all containing ellagitannins)	300 mL (wine); 250 g (strawberries); 225 g (raspberries); 35 g (walnuts)	Acute crossover	40 healthy volunteers	LC-MS/MS (Targeted)	Urine	• Urolithin B derivatives (glucuronide more than aglycone)	(64)
Wine	Wine vs. vodka (diluted in lemon-flavoured water) vs. dealcoholized wine vs. placebo (lemon-flavoured water)	147 mL	Acute, randomized, controlled, crossover	28 healthy men	Method not reported (Targeted)	Urine	• Hydroxytyrosol • 3'-Dihydroxyphenylacetic acid • Homovanillic acid • 4-Hydroxyphenylacetic acid	(65)
Wine	Wine (alcohol consumption) vs. no wine (alcohol abstinent)	1 glass/day for women (16 g ethanol); 2 glasses/day for men (32 g ethanol)	3-month, randomized, controlled, parallel	44 healthy volunteers	LC-MS/MS (Targeted)	Hair	• Ethyl glucuronide	(66)
Wine	Red wine vs. red grape juice	400 mL	Acute crossover	9 healthy volunteers	HPLC-UV-VIS (Targeted)	Plasma, urine	• Total anthocyanins • Cyanidin-3-glucuronide • Delphinidin-3-glucuronide • Malvidin-3-glucuronide • Peonidin-3-glucuronide • Petunidin-3-glucuronide	(67)
Wine	Red wine vs. delacoholized red wine vs. red grape juice	500 mL	Acute, randomized, crossover	6 healthy men	HPLC-PDA (Targeted)	Plasma, urine	• Malvidin-3-glucoside	(68)
Wine	Wine vs. mature whisky vs. new whisky	100 mL	Acute, randomized, crossover	9 healthy volunteers	Folin Ciocalteu method (Targeted)	Plasma, urine	• Total phenols (gallic acid equivalents)	(69)
Wine	Red wine vs. Red grape juice	400 mL	Acute, randomized, crossover	9 healthy volunteers	HPLC-UV-VIS (Targeted)	Plasma, urine	• Total anthocyanins • Cyanidin-3-glucuronide • Delphinidin-3-glucuronide • Malvidin-3-glucuronide • Peonidin-3-glucuronide • Petunidin-3-glucuronide	(70)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Wine	Wine	250 mL	Acute intervention	7 healthy volunteers	LC-MS/MS (Targeted)	Urine	• Ethyl glucuronide	(71)
Wine	Listerine mouth rinse vs. non-alcoholic wine vs. vodka	4.2 to 7.5 dL (wine); 3.75 mL (vodka)	Acute parallel	12 healthy volunteers	HPLC-MS/MS, HPLC-UV (Targeted)	Oral fluid, whole blood, urine	• Ethyl glucuronide • Ethyl sulfate	(72)
Wine	Pinot noir red wine vs. Cabernet sauvignon red wine vs. dry semillon white wine vs. sauvignon blanc white wine vs. absolute alcohol in water	0.3 g/kg body weight ethanol	Acute, randomized, crossover	108 volunteers with previous illness	GLC (Targeted)	Blood	• Ethanol	(73)
Rice wine	Japanese rice wine (sake) vs. water	0.4 g/kg body weight ethanol	Acute intervention	63 healthy men	GC-FID (Targeted)	Breath, blood	• Alcohol • Acetaldehyde	(74)
Rice wine	Sake vs. rice wine	100 mL (sake); 50 mL (rice wine)	Acute intervention	2 healthy volunteers	1H NMR (Targeted)	Urine	• Ethyl glucoside	(75)
Yoghurt	Yoghurt vs. milk	800 g	Acute, randomized, double-blind, crossover	14 healthy men	GC-MS (Targeted)	Serum	• Lactose • Galactose	(76)
Yoghurt	Probiotic yoghurt with *Bifidobacterium animalis* subsp. *lactis* LKM512 or placebo yoghurt	100 g/day	4-week, double-blind, placebo-controlled, crossover	10 adults with moderate atopic dermatitis	HPLC-Scanning FLD (fecal polyamines); HPLC-Differential Refractometer (fecal SCFAs) (Targeted)	Feces	• Butyrate vs baseline (no increases for other SCFAs measured, acetate, propionate, isobutyrate, valerate, and isovaleate) • Spermidine (also some with increased putrescine, but spermine levels not detected)	(77)
Yoghurt	Yoghurt consumers vs. non-consumers	>200 g/day (consumers), 0 g/day (non-consumers) (from 24-h food records)	Free-living cohort	30 (consumers) ad 21 (non-consumers) from the	GC (detector not specified) (SCFAs); GLC (for bile acids) (Targeted)	Feces	• No significant differences in fecal SCFA (total, acetate, propionate, butyrate, *iso*-acids, valerate + caproate) • No significant differences in neutral bile acids (cholesterol, coprostanol, cholestanol, coprostanone, beta-sitosterol, copro-beta-sistosterol)	(78)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
				SU.VI.MAX cohort			• Increase in 7-keto-lithocholic (no significant differences in other acidic bile acids cholic, deoxycholic, ursocholic, 7-keto-deoxycholic, chenodeoxycholic, lithocholic, ursodeoxycholic, 7-keto-lithocholic, sum)	
Yoghurt	Probiotic yoghurt with *Bifidobacterium animalis* subsp. *lactis* LKM512 vs. placebo yoghurt	100 g/day	2-week, placebo-controlled, crossover	7 healthy adults	HPLC-Scanning FLD (fecal polyamines); HPLC-Differential Refractometer (fecal SCFAs) (Targeted)	Feces	• Spermidine (putrescine not increased, spermine levels not detected) • No significant changes in lactate or acetate	(79)
Yoghurt	Fresh yoghurt (>10^7 CFU) vs. heated yoghurt (<10^2 CFU)	500 g/day	15-day, randomized	12 healthy men with lactose malabsorption and 12 healthy men without lactose malabsoption	HS-GC-FID (SCFAs) (Targeted)	Plasma	• Butyrate (in volunteers without lactose malabsorption for fresh yoghurt vs. heated) • Propionate (in volunteers with lactose malabsorption for fresh yoghurt vs. baseline) • No change in acetate	(80)
Yoghurt	Whole milk, commercial unflavoured yoghurt, heated yoghurt	400 mL (milk); 450 g (yoghurts)	Acute, randomized, crossover	8 healthy volunteers with lactose malabsorption	Enzymatic assay (Targeted)	Ileal fluid	• Lactose (higher after heated yoghurt than yoghurt) • Galactose (not signficant) • Glucose (not significant) • Hexoses (higher after heated yoghurt than yoghurt)	(81)
Cheese, yoghurt (general dairy)	Total dairy intake	Quintiles of total dairy intake: Q1: 0-2.07, Q2: 2.1-4.1, Q3: 4.13-6.65, Q4: 6.69-9.73, Q5: 9.8-31.08 (from FFQ)	Association study	659 volunteers without diabetes from the IRAS cohort	GC-FID (Targeted)	Serum	• Pentadecanoic acid (15:0) • Trans-palmitoleic acid (trans 16:1n-7) was not considered to be a specific marker for total dairy intake	(82)
Cheese, yoghurt (general dairy)	Dairy fat intake	Continuous levels of dairy fat intake (from 1 week weighted diet records and FFQ)	Association study	81 healthy women	GC with (Targeted)	Subcutaneous adipose tissue	• Pentadecanoic acid (15:0) • Heptadecanoic acid (17:0)	(83)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cheese, yoghurt (general dairy)	Natural yoghurt, cheddar cheese, semi-skimmed milk, water (dairy as snack)	410 mL	Acute, randomized, crossover	40 overweight men	GC-FID (Targeted)	Plasma	• Amino acids (alpha-amino butyric acid, Ala, Asn, cysteine, Gly, Glu, His, Ile, Leu, Lys, Met, Orn, phe, Pro, Ser, Thr, Trp, Try, Val)	(84)
Cheese, yoghurt (general dairy)	Dairy: Milk (1% fat), yoghurt (1.5% fat), cheese (34% fat) Control: fruit and vegetable juice, cashews and a cookie	3 servings/day (375 mL milk/day, 175 yoghurt/day, 30 g cheese/day)	4 week, randomized, free-living, multi-center, crossover	124 healthy volunteers	GC-FID (Targeted)	Plasma	• Pentadecanoic acid (15:0) • Heptadecanoic acid (17:0) • Trend towards higher cis-9, trans-11-18:2n-6, p=0.06) • Total SFA • 18:3n-6 • 22:1n-9 • SFA:MUFA and SFA:PUFA	(85)
Cheese, yoghurt (general dairy)	Total dairy, high-fat dairy, low-fat dairy, milk, cream, yoghurt, cheese, butter	Continuous levels of total dairy, high-fat dairy, low-fat dairy, milk, cream, yoghurt, cheese, butter (from FFQ)	Association study	334 control and 1054 intervention volunteers from Food4Me	GC-FID (Targeted)	Dried blood spots	• Pentadecanoic acid (15:0) • Heptadecanoic acid (17:0)	(86)
Cheese, yoghurt (general dairy)	Meat, dairy food, egg, and fish	Quartiles/levels of milk and dairy products, milk, cheese, total meat, red meat, processed meat, white meat, fis hand shellfish, and eggs and egg products (from 24-h recalls)	Association study	271 participants of the Second Bavarian Food Consumptio n Survey	LC-MS (Targeted)	Plasma	• Trimethylamine-N-oxide with milk and dairy products, milk (not for other food groups) • No associations for betaine or choline	(87)
Cheese, yoghurt	Dairy (total dairy, milk, cheese, yoghurt, cream/butter) intake	Frequency of dairy consumption from never/ <1 time/month to ≥6 times/day (from FFQ)	Association study	2205 volunteers from the Framingha m Health Study Offspring cohort and 866 volunteers from the Gen3 cohort	HILIC- and lipid-LC-MS and MS/MS (Targeted and Untargeted)	Plasma	• Cheese: C46:0 triacylglycerol, C54:4 triacylglycerol, C54:5 triacylglycerol, C54:6 triacylglycerol • Yoghurt: C20:5 cholesterol ester	(88)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cheese, yoghurt, fermented milk (general dairy)	Total milk products, cream, cheese, fermented milk, total milk, ice cream	Quartiles of of total milk products, cream, cheese, fermented milk, total milk, ice cream, and 15:0 and 17:0 (from FFQ)	Prospective case-control nested within larger cohort NSHDS	444 cases of myocardial infarction and 556 controls	GLC (Targeted)	Plasma	• Pentadecanoic acid (15:0) • Heptadecanoic acid (17:0)	(89)
Cheese, soy sauce	Soy sauce with a meal (not further specified) or Appenzeller cheese	9 mL soy sauce; 10 g cheese	Acute intervention	4 volunteers (cheese); 3 volunteers (soy sauce)	HPLC-IS-MS (Targeted)	Urine	• 4-methylspinacemine (4-methyl-4,5,6,7-tetrahydro-1H-imidazo-[4,5-c]pyridine) • 1,4-dimethylspinacemine	(90)
Cheese	Probiotic cheese with *L. plantarum* TENSIA vs. control cheese	50 g/day	3-week, randomized, double-blind, placebo-controlled, parallel	25 hospitalized patients	GC-FID (Targeted)	Urine	• Putrescine and acetylated putrescine in control group decreased vs baseline (no significant differences in other polyamines, tyramine or acetylated spermidine) • Extent of change is significantly higher for probiotic cheese vs control for putrescine and acetylated putrescine	(91)
Cheese	Cheese (from cows with linseed oil added tot heir diet) vs. control cheese	3 X 50 g/week	4-week, randomized, double-blind, crossover	30 healthy, free-living volunteers	GC-FID (Targeted)	Serum	• C18:0 in test cheese group vs baseline • No other significant differences in serum fatty acids compared to baseline for either group (12:0, 14:0, 16:0, 18:0, 16:1, 18:1, 18:2, 20:4)	(92)
Soy	Untreated soymilk vs. beta-glucosidase soymilk vs. fermented soymilk	100 mL	7-day crossover	12 healthy volunteers	LC-MS (Targeted)	Serum, urine	• Isoflavones (daidzein, genistein, glycitein)	(93)
Soy	Breakfast casseroles containing tofu, tempeh, cooked soybeans, or texturized vegetable protein	30 g (texturized vegetable protein); 100 g (tempeh); 100 g (cooked soybeans); 300 g tofu	Acute, randomized, crossover	10 healthy women	HPLC with variable wavelength detector (Targeted)	Plasma, urine, feces	• Isoflavones (daidzein, genistein)	(94)
Soy	Breakfast with natto and spinach vs. detergent-solubilized K1 vs. low-vitamin K diet	200 g (natto) and 400 g (spinach)	Acute crossover	6 healthy men	HPLC-FLD (Targeted)	Serum	• Vitamin K2 (menaquinones); higher than vitamin K1 (phylloquinones in spinach)	(95)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Soy	Fermented soy vs. non-fermented soy	112 g (tempeh); 125 g (soybeans)	9-day, randomized, crossover	22 healthy men	GC-MS (Targeted)	Urine	• Isoflavoids (equol, O-desmethylangolensin, daidzein, genistein), daidzein and genistein recover greater with tempeh diet • Lignans (enterolactone, enterodiol) decreased with soy intake	(96)
Soy	Fermented soymilk containing *Bifodibacterium animalis* Bb-12 vs. non-fermented soymilk	200 mL/day, containing 20, 40, or 80 mg isoflavone/200 mL	14-day, randomized, double-blind, crossover	16 healthy postmenopausal women	HPLC-UV-VIS (Targeted)	Urine	• Isoflavones (genistein, daidzein, glycitein) aglycones, and beta-glucoside isomers	(97)
							• Equol (tendency to increase in the fermented group over time but did not reach significance)	(98)
Soy	Isogen capsules vs. soymilk vs. fermented soybeans	84.8 mg (isogen); 43.8 g (fermented soybeans); 600 mL (soymilk); standardised to 64.8 mg isoflavones	Acute, randomized, controlled, parallel	26 healthy women	HPLC (detector not specified) (Targeted)	Plasma, urine	• Isoflavones (genistein, daidzein) higher urinary recovery in fermented group, longer plasma half-life in fermented and isogen groups	(99)
Soy	Tofu, natto (fermented soybean), soy milk, soy isoflavone supplement	180 g (tofu), 100 g (natto), 300 g (soymilk), 1 supplement, all standardized to 75 mg isoflavones	1-day intervention	20 healthy women (9 equol producers and 11 non-producers)	HPLC-UV (Targeted)	Urine	• Isoflavones (genistein, daidzein)	(100)
Soy	Fermented soy powder (aglycone-rich) with *Aspergillus oryzae* vs. non-fermented soy powder (glycoside-rich)	23 g (standardized to 95 umol isoflavones)	Acute, randomized, double-blind, crossover	11 healthy postmenopausal women	LC-MS/MS (Targeted)	Serum, urine	• Isoflavones (total) increased bioavailability and urinary excretion; trend towards increased in daidzein, genistein, and glycitein as well, no significant difference in equol	(101)
Soy	Stinky tofu	146 g	Acute intervention	36 healthy volunteers (18 equol producers, 18 non-producers)	HPLC-UV (Targeted)	Urine	• S-equol, similar rates of excretion in producers and non-producers • Daidzein and dihydrodaidzein • Total daidzein (daidzein, dihydrodaidzein, S-equol, O-desmethylangolensin)	(102)
Soy	Fermented soymilk with *Lactobacillus casei* Shirota	100 mL	Acute, randomized, double-blind, placebo-	7 healthy postmenopausal women	LC-MS/MS (Targeted)	Serum	• Isoflavones (genistein, daidzein, glycitein, dihydrodaidzein, O-desmethylangolensin, equol)	(103)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	(containing aglycones) vs. placebo soymilk (no aglycones)		controlled, crossover					
Soy	Natto (fermented with *Bacillus natto*)	Natto consumption frequency (questionnaire); 80 g in acute intervention	Cross-sectional, and acute intervention	Group 1: 49 postmenopausal women from Tokyo (8 also participated in acute intervention) Group 2: 25 postmenopausal women from Hiroshima Group 3: 31 postmenopausal British women	HPLC-FLD (Targeted)	Serum	• Vitamin K2 (menaquinone-7) • Phylloquinone (slightly higher in Japanese than British women) • Menaquinone-4 (below limit of detection in most women)	(104)
Soy	Soya milk vs. miso soup	250 mL (soymilk); 31 g Hacco miso; standardized to 48 mg isoflavones	1-day or 2 non-consecutive days, randomized, crossover	21 healthy women	LC-MS (Targeted)	Urine	• Isoflavones (daidzein, genistein, equol)	(105)
Soy	Fermented soy (tempeh) vs. non-fermented soy (soybean)	112 g (tempeh); 125 g (soybean)	9-day, randomized, controlled, crossover	17 healthy men	GC-MS (Targeted)	Urine	• Isoflavones (daidzein, genistein) higher excretion for fermented group (although fermentation decreased isoflavone content of the soy product)	(106)
Soy	Soy flour vs. fermented soybean paste	12.2 g (soy flour); 52 g (soybean paste); standardized to 20 mg isoflavones	Acute, randomized, controlled, crossover	10 healthy volunters	LC-ESI-MS/MS (Targeted)	Urine	• 20 isoflavone metabolites detected, consisting of daidzein and genistein glucuronides and sulfoglucuronides, daidzein sulfate, equol glucuronide, and ODMA glucuronides) • Isoflavones (especially glucuronides of daidzein, genistein) higher in fermented group	(107)
Soy	Natto fermented with *Bacillus subtilis,* double-boiled natto with short interval, vitamin K syrup	50 g/day	3-day intervention with untreated natto; acute, crossover with natto, boiled natto,	32 healthy volunteers	HPLC (detector not specified) (Targeted)	Plasma	• Menaquinone-7 (increased following natto consumption; negligible levels of phylloquinone and menaquinone-4) • Lower levels of menaquinone-7 following boiled natto consumption compared to regular natto and syrup	(108)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
			and vitamin K syrup					
Soy	Fermented soybean soup with *Aspergillus oryzae* vs. placebo soup	20 g powder with water; standardized to 24 mg isoflavones/day	4-week, randomized, double-blind, placebo-controlled, parallel	65 healthy postmenopausal women	HPLC-UV (Targeted)	Urine	• Isoflavones (daidzein, genistein, glycitein), total isoflavoens, especially daidzein and glycitein, were higher in fermented group	(109)
Soy	Soybean milk, fried bean curd puff, fresh bean curd, soybeans, fermented bean curd, and other bean foods	Quartiles of soy product consumption, including soybean milk, fried bean curd puff, fresh bean curd, soybeans, fermented bean curd, and other bean foods (from FFQ)	Case-control nested within a large randomized trial of breast self-examination among textile workers in Shanghai	1823 women (1590 women without breast cancer, and 233 women with breast cancer)	LC-coularray and LC-MS (Targeted)	Serum	• Isoflavones (daidzein, genistein)	(110)
Soy	Regular natto vs. reinforced natto 1 vs. reinforced natto 2	50 g (775 ug/100g MK7; 1298 ug/100g MK7; 1765 ug/100g MK7)	7-day crossover	8 healthy men	HPLC (detector not specified) (Targeted)	Serum	• Vitamin K2 (menaquinone-7)	(111)
	Rare intake of natto vs. occasional intake vs. frequent intake	~0 (rare); a few times a month (occasional), a few times a week (frequent) (from food record)	Cross-sectional	134 healthy volunteers				
Soy	Biscuits with fermented soybean meal vs. biscuits with soybean meal	75 g; dose equivalent to 0.44 mg/kg body weight of isoflavones	Acute, randomized, double-blind, crossover	18 healthy volunteers	HPLC-DAD-FLD (Targeted)	Urine	• Glycitein • Daidzein • Genistein • Dihydrodaidzein • O-demethylangolensin • Dihydrogenistein • 6-hydroxy-O-demethylangolensin • Equol • Total aglycones • Total colonic metabolites	(112)

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Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Soy	Testmeals with fermented soybean vs. non-fermented soybean	33 g of soybean	Acute (2-dose), randomized, controlled, crossover	12 healthy volunteers	UPLC-DAD-QTOF-MS (Targeted)	Plasma, urine	• Soy isoflavones: genistein 5-O-glucoside, daidzein 7-O-glucoside (daidzin), glycitein 7-O-glucoside (glycitin), genistein 7-O-glucoside (genistin), daidzein 4′-O-(6″-O-malonyl)glucoside, genistein 5-O-(6″-O-malonyl)glucoside, daidzein 7-O-(4″-O-malonyl)glucoside, glycitein 7-O-(4″-O-malonyl)glucoside, daidzein 7-O-(6″-O-malonyl)glucoside, glycitein 7-O-(6″-O-malonyl)glucoside, daidzein 7-O-(6″-O-succinoyl)glucoside, daidzein 7-O-(6″-O-acetyl)glucoside, genistein 4′-O-(6″-O-malonyl)glucoside, genistein 7-O-(4″-O-malonyl)glucoside, glycitein 7-O-(6″-O-acetyl)glucoside, genistein 7-O-(6″-O-malonyl)glucoside, genistein 4′-O-(6″-O-acetyl)glucoside, daidzein, genistein 7-O-(6″-O-succinoyl)glucoside glycitein, genistein 7-O-(6″-O-acetyl)glucoside genistein, Sum of glucoside forms, simple glucoside, acetyl glucoside, succinyl glucoside, malonyl glucoside)	(113)
Soy	Fermented soybean paste (Cheonggukjang)	50 g/kg body weight	Acute intervention	48 healthy men	UPLC-QTOF-MS (Targeted)	Plasma	• Isoflavone metabolites: Daidzein, Genistein, Glycitein, 3-hydroxydaidzein, 2-hydroxygenistein, Daidzein 4'-glucuronide, Daidzein 7-glucuronide, Daidzein diglucuronide, Daidzein 4'-sulfate, Daidzein 7-sulfate 4'-glucuronide, Genistein 7-glucuronide, Genistein diglucuronide, Genistein-7-glucuronide-4'-sulfate, Genistein 4'-sulfate, Genistein-7-sulfate, Dihydrogenistein, Dihydrodaidzein sulfate, Equol-7-glucuronide, Equol-4-sulfate, 5-hydroxy equol, O-Desmethylangolensin)	(114)
Bread	Whole-grain and fibre-rich rye bread vs. refined wheat bread	174 g/day (wholegrain products); 188 g/day (refined white breads)	12-week, randomized, parallel	51 volunteers with metabolic syndrome	GC-MS (Targeted)	Plasma	• Alkylrecorsinols (AR homologs C17-25:0)	(115)
Bread	Wholegrain wheat and rye	Continuous intake of various wholegrains (from FFQ)	Association study	360 postmenopausal women from the Danish Diet, Cancer and Health Study	GC-MS (Targeted)	Plasma	• Alkylrecorsinols (AR homologs C17-25:0)	(116)
Bread	High-fiber rye bread	198 g (containing 45 mg ARs) and 21 g butter	Acute intervention	15 healthy volunteers	HPLC-CEAD (Targeted)	Plasma	• Alkylrecorsinol metabolites DHBA and DHPPA	(117)
Bread	Whole grain breads	Total daily whole grain intake (whole grain soft bread + dark	Association	20 free-living women from the Swedish	GC-MS (Targeted)	Plasma, subcutaneous adipose tissue	• Alkylrecorsinols (AR homologs C17-25:0)	(118)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
		crisp bread) (from FFQ)		Mammography Cohort				
Bread	Wholegrain rye porridge vs. refined wheat bread	2 wholegrain rye porridges (40/55 g), 3 rye porridges with different inulin (55 g); refined wheat bread (55 g)	Acute, randomized, crossover	21 healthy volunteers	NMR (36 plasma metaboites); GC-MS (short chain fatty acids) (Targeted)	Plasma	• Valine, leucine, isoleucine, lysine, phenylalanine, 2-oxoisocaproate • Acetate, butyrate, propionate, acetoacetate, 3-hydroxybutyrate	(119)
Bread	Rye bran bread products vs. control wheat bread products	250 g	6-week, randomized, crossover	18 healthy postmenopausal women	HPLC-DAD (Targeted)	Urine	• Ferulic acid	(120)
Bread	Wheat bread vs. fresh pasta (both enriched with wheat bran)	132 g (bread); 119 g (pasta)	Acute, randomized, crossover	9 healthy men	GC-MS (Targeted)	Plasma	• L-Isoleucine, L-leucine, lactic acid, fructose, xyloe, arabinose, 2,4-dihydroxybutanoic acid, L-phenylalanine, L-proline (treatment X time effect) • 1,2-diglyceride, 1-methylhistidine, urea, sitosterol, glyceric acid, phosphate, fumaric acid (treatment effect) • C14:0, C17, glycerol, beta-alanine, L-valine, L-tryptophan, L-tyrosine, inositol, succinic acid, citric acid, L-ornithine, mannose, 2-hydroxypiperidine, iminodiacetic acid, ribulose/xylulose (treatment and time effect)	(121)
Bread	Wholegrain bread vs. bread enriched with aleurone fraction	94 g (wholegrain bread had 87 mg of ferulic acid, while aleurone bread had 43 mg)	Acute, single-blind, randomized, crossover	15 healthy volunteers	U(H)PLC-MS and MS/MS (Targeted)	Urine, plasma	• Ferulic acid metabolites (especially ferulic acid-4'-O-sulfate, dihydroferulic acid-4'-O-sulfate, dihydroferulic acid-O-glucuronide)	(122)
Bread	Wholegrain wheat crisp bread vs. wholegrain rye crisp bread	100 g/day	1-week, randomized, crossover	15 healthy volunteers	GC-MS (ARs), automatic fluoroimmunoassay (enterolactone) (Targeted)	Plasma, erythrocytes, lipoproteins (ARs), serum (enterolactone)	• Alkylrecorsinols (AR homologs C17-21:0) • Enterolactone	(123)
Bread	Wholegrain rye and wheat bread vs. no wholegrain rye and wheat products (in habitual diet)	1. Habitual diet of wholegrain rye bread (3-5 pieces/day) and wholegrain wheat bread (2 pieces/day)	1. 1 week avoidance, 1 week habitual diet 2. 2 weeks of habitual diet	1. 4 healthy volunteers 2. 4 healthy voluntters	GC-MS (Targeted)	Plasma, erythrocytes	• Alkylrecorsinols (AR homologs C17-25:0)	(124)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
		2. Habitual diet and wholegrain rye bread (2 pieces/day) and wholegrain wheat bread (2 pieces/day) 3. Gluten-free diet with no wheat, rye, or barley products	3. 2 weeks of gluten-free diet	3. 1 volunteer				
Bread	High-fiber rye bread	198 g (containing 100 mg ARs) and 21 g butter	Acute intervention	15 healthy volunteers	HPLC-CEAD (Targeted)	Urine	• Alkylrecorsinol metabolites DHBA and DHPPA	(125)
Bread	Bread, rye bread, refined wheat bread, bran-seed bread	Continuous intake of bread, rye bread, refined wheat bread, bran-seed bread (from 3-day food records)	Association study	31 free-living men with prostate cancer and 91 non-cancer control men	HPLC-CEAD (Targeted)	Plasma (DHPPA), urine (DHBA and DHPPA)	• Alkylrecorsinol metabolites DHBA and DHPPA	(126)
Bread	Wholegrain products	Continuous intake of wholegrain products, including rye bread, wholegrain bread, rolled oats and muesli, and crispbread (from FFQ)	Association study	43 893 volunteers from the Danish Diet, Cancer, and Health cohort (ARs were measured in subset of 516 volunteers)	GC-MS (Targeted)	Plasma	• Alkylrecorsinols (AR homologs C17-25:0)	(127)
Bread	Wholegrain wheat vs. refined wheat	70 g/day biscuits (wholegrain); 33 g/day crackers and 27 g/day toasted bread (refined grain)	8-week, randomized, placebo-crontrolled, parallel	80 healthy obese/over weight volunteers	HPLC-MS/MS (Targeted)	Serum, urine, feces	• Phenolic acids (dihydroferulic acid in serum, ferulic acid in feces following wholegrain wheat)	(128)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Bread	Refined-grain wheat bread vs. wholegrain wheat bread	196 g (refined grain); 208 g (wholegrain), both with 21 g butter	Acute, crossover	12 healthy volunteers	LC-MS (Targeted)	Urine	• Alkylrecorsinol metabolites (3,5-DHBA glycine, 3,5-DHPPTA, 3,5-DHBA, 3,5-DHPPA)	(129)
Bread	Wholegrain rye bread vs. low-fiber wheat bread	214 g (rye); 178 (wheat)	8-week, randomized, crossover	39 healthy postmenopausal women	GC-MS (ARs); fluoroimmunoassay (enterolactone) (Targeted)	Plasma	• Alkylrecorsinols • Enterolactone	(130)
Bread	White wheat flour soft bread and white wheat flour crispbread vs. rye bran-enriched soft bread and whole grain rye crisp bread	142.8 g/day and 92.4 g/day; 180.6 g/day and 91.0 g/day	2-week, randomized, crossover	10 volunteers with previous proctocolectomy for ulcerative colitis	GC-MS (Targeted)	Ileal effluent	• Alkylrecorsinols (AR homologs C17-25:0)	(131)
Bread	Sourdough fermented bread vs. traditional sourdough bread vs. baker's yeast bread	215 mL (mimicking chewing and homogenisation of the bread portion + 160 mL water)	Acute, randomized, double-blind, crossover	36 healthy volunteers	Biochrom 30 series Amino Acid Analyzer (Targeted)	Plasma	• Total free amino acids	(132)
Bread	Rye-based bread vs. white wheat bread	75 g	3-day, randomized, controlled, crossover	38 healthy volunteers	GC (detector not specified) (Targeted)	Plasma	• Acetate • Butyrate • Total SCFAs	(133)
Cocoa	Sugar-free, flavanol-rich cocoa alone vs. low-dose sugar + cocoa vs. high-dose sugar + cocoa	0.125 g/kg body weight (cocoa); 8.75 kJ/kg (low-dose sugar); 17.5 kJ/kg (high-dose sugar)	Acute crossover	6 healthy volunteers	HPLC-coulometric multiple-array detection (Targeted)	Plasma	• Total flavonols (epicatechin + catechin)	(134)
	Sugar-free, flavanol-rich cocoa alone vs. bread+ cocoa vs. steak + cocoa vs. butter + cocoa	0.125 g/kg body weight (cocoa); 17.5 kJ/kg (foods)					• Total flavonols (epicatechin + catechin)	

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	(macronutrient-rich foods)							
	Sugar-free, flavanol-rich cocoa alone vs. bread + cocoa vs. milk + cocoa vs. grapefruit juice + cocoa	0.125 g/kg body weight (cocoa); 17.5 kJ/kg (foods)					• Total flavonols (epicatechin + catechin)	
	Water + cocoa vs. water + cocoa + Famotidine	0.125 g/kg body weight (cocoa); 20 mg (Famotidine)					• No difference in flavonols	
Cocoa	Semi-sweet chocolate (M&Ms chocolate mink baking bits) vs. vanilla milk chips	80 g	Acute, parallel	13 healthy volunteers	HPLC-Coulochem II detector (Targeted)	Plasma	• Catechin • Epicatechin	(135)
Cocoa	Nestle Noir 70% dark chocolate	100 g	Acute intervention	5 healthy volunteers	LC-MS/MS (Targeted)	Plasma, urine	• (-)-Epicatechin-3'-beta-D-glucuronide • (-)-Epicatechin-3'-sulfate • 3'-O-methyl epicatechin sulfates	(136)
Cocoa	Cocoa powder + skimmed milk vs. skimmed milk	20 g/day with 250 mL skimmed milk; 500 mL/day skim milk	4-week, randomized, controlled, crossover	42 healthy volunteers	LC-MS/MS (Targeted)	Plasma, urine	• (-)-Epicatechin • (-)-Epicatechin glucuronides and sulfates • O-methyl-epicatechin glucuronides and sulfates • DHPV glucuronides and sulfates • MHPV glucuronides and sulfates • Vanillic acid • 3,4-Dihydroxyphenylacetic acid • 3-Hydroxyphenylacetic acid	(137)
Cocoa	Cocoa powder + water vs. cocoa powder + whole milk vs. whole milk	40 g (cocoa powder) in 250 mL (milk or water)	Acute, randomized, crossover	21 healthy volunteers	HPLC-MS/MS (Targeted)	Urine	• (-)-Epicatechin glucuronide • (-)-Epicatechin sulfates	(138)
Cocoa	Dark chocolate vs. high sucrose chocolate vs. high milk protein chocolate vs.	40 g or 250 mL (for drinks)	Acute, randomized, crossover	6 healthy volunteers	HPLC-EAD (Targeted)	Serum	• Epicatechin, catechin, and glucuronide and sulfate conjugates	(139)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	sucrose milk protein chocolate drink vs. non-nutritive sweetner milk protein chocolate drink							
Cocoa	Flavonoid-rich dark chocolate (72%) vs. flavonoid-free placebo (dyed) white chocolate	50 g	Acute, randomized, controlled, parallel	65 healthy men	HPLC-EAD (Targeted)	Plasma	• Epicatechin	(140)
Cocoa	High-flavanol chocolate with mannitol vs. high-flavanol chocolate with sucrose vs. low-flavanol chocolate with sucrose	25 g; standardized to macro and micronutrients, theobromine, and caffeine	Acute, randomized, double-blind, crossover	15 healthy volunteers	HPLC-FLD/UV (Targeted)	Plasma	• Total cocoa flavanols (epicatechin, catechin, procyanidin oligomers) • 3' and 4'-O-methylated epicatechins	(141)
Cocoa	Cocoa beverage vs. milk	40 g cocoa powder in 250 mL milk; 250 mL milk	Acute, randomized, controlled, crossover	36 healthy volunteers	Folin-Ciocalteu Assay (modified) (Targeted)	Urine	• Total polyphenols (+-catecgub equivalents)	(142)
Cocoa	Cocoa with water	40 g cocoa powder in 250 mL water	Acute intervention	21 healthy volunteers	LC-MS/MS (Targeted)	Urine	• Caffeic acid • Ferulic acid • 3-hydroxyphenylacetic acid • Vanillic acid • 3-hydroxybenzoic acid • 4-hydroxyhippuric acid • Hippuric acid • (-)-Epicatechin • Procyanidin B2	(143)
Cocoa	Dark chocolate (70%) vs. white chocolate	45 g	2-week, controlled, parallel	20 healthy volunteers	HPLC-DAD (Targeted)	Plasma	• (-)-Epicatechin	(144)
Cocoa	Cocoa beverage with milk vs. cocoa beverage with water	10 g cocoa power in 250 mL milk or water	Acute crossover	9 healthy volunteers	HPLC-PDA, HPLC-MS (Targeted)	Plasma, urine	• (Epi)catechin-O-sulfate • (-)-Epicatechin-O-glucuronide • (Epi)catechin-O-sulfate • O-Methyl-(epi)catechin-O-sulfate	(145)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cocoa	Hershey's milk chocolate	113 g	Acute intervention	6 nursing mothers	HPLC (detector not specified) (Targeted)	Plasma, saliva, breast milk	• Theobromine	(146)
Cocoa	Dark chocolate vs. white chocolate	6.3 g/day	18-week, randomized, controlled, parallel	44 healthy volunteers	LC-MS/MS (Targeted)	Plasma	• Epicatechin • Catechin • Procyanidin B2 • Procyanidin B2 galate	(147)
Cocoa	Cocoa powder with no added methylxanthine vs. cocoa powder enriched with methylxanthine	15 g dissolved in 200 mL (milk)	Acute, randomized, controlled, crossover	13 healthy volunteers	HPLC-DAD, LC-QTOF, LC-DAD (Targeted)	Plasma, urine	• Theobromine • Caffeine • Paraxanthine • Theophylline • Methylxanthines (1-, 3-, 7, 1,7-, 3,7-, 1,3-, 1,3,7-) • Methyluric acid (1-, 1,3-, 1,7-, 3,7-, 1,3,7-)	(148)
Cocoa	Cocoa in skimmed milk vs. skimmed milk	40 g/day (cocoa powder) in 500 mL (milk)	4-week, randomized, crossover	42 volunteers with high CV risk	LC-MS/MS (Targeted)	Urine	• (-)-Epicatechin glucuronides and sulfates • O-Methyl-epicatechin glucuronides and sulfates • 5-(3-Methyoxy-4-hydroxyphenyl)-gamma-valerolactone glucuronides and sulfates • 5-(3,4-Methyoxy-4-hydroxyphenyl)-gamma-valerolactone • 3-Hydroxyphenylacetic acid • 3,4-Dihydroxyphenylacetic acid • Vanillic acid	(149)
Cocoa	Dark chocolate (85%) vs. milk chocolate (<35%)	40 g	Acute, single-blind, crossover	20 healthy volunteers and 20 smokers	HPLC-UV (Targeted)	Serum	• Epicatechin	(150)
Cocoa	Cocoa beverage	0.375 g cocoa/kg body weight in 300 mL water	Acute intervention	5 healthy volunteers	HPLC-CEAD, LC-MS/MS (Targeted)	Plasma	• Procyanidin dimer B2 [epicatechin-4beta-8)-epicatechin] • Catechin • Epicatechin	(151)
Cocoa	Flavanoid-enriched chocolate bars vs. placebo	27 g/day with 90 mg catechin/day	1-year, randomized, controlled, parallel	93 postmenopausal women with type II diabetes	LC-MS/MS (Targeted)	Urine	• Epicatechin monoglucuronide • Methylepicatechin monoglucuronide • Epicatechin monosulfate • Methylepicatechin sulfate • Epicatechin monosulfate monoglucuronide	(152)
Cocoa	Cocoa in whole milk vs. cocoa in water vs. milk	40 g (cocoa powder) in 250 mL (milk or water)	Acute, randomized, crossover	21 healthy volunteers	LC-MS/MS (Targeted)	Plasma	• (-)-Epicatechin glucuronide	(153)
Cocoa	Cocoa beverage	Powder in 500 mL water; 10.7 mg cocoa	Acute intervention (X2)	7 healthy young volunteers	HPLC-FLD/UV/ECD (Targeted)	Plasma, urine	• (-)-Epicatechin-3'-beta-D-glucuronide • (-)-Epicatechin-3'-sulfate • 3'-O-Methyl-(-)-epicatechin-5-sulfate • 3'-O-Methyl-(-)-epicatechin-7-sulfate	(154)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
		flavanols/kg body weight						
	Cocoa beverage vs. acetaminophen in beverage	Powder in 500 mL water; 5.3 and 10.7 mg cocoa flavanols/kg body weight; 2X 500 mg acetaminophen in water	Acute, randomized, crossover	20 young healthy men and 20 elderly healthy men			• (-)-Epicatechin-3'-beta-D-glucuronide • (-)-Epicatechin-3'-sulfate • 3'-O-Methyl-(-)-epicatechin-5-sulfate • 3'-O-Methyl-(-)-epicatechin-7-sulfate	
Cocoa	High-flavanol cocoa drink vs. low-flavanol cocoa drink	Powder in 300 mL water; 917 mg or 37 mg cocoa flavanols	Acute, randomized, double-blind, crossover	10 healthy men	HPLC-MS (Targeted)	Plasma	• (-)-Epicatechin • Catechin • 4'-Methyl-epicatechin • Epicatechin-O-beta-D-glucuronide • 4'-O-Methyl-epicatechin-O-beta-D-glucuronide (highest)	(155)
Cocoa	Dark chocolate	100 g	Acute intervention	Healthy volunteers (number not reported)	LC-MS/MS (Targeted)	Urine	• 3'-O-methyl-(-)-epicatechin-5/7-O-sulfate • 4'-O-methyl-(-)-epicatechin-5/7-O-sulfate	(156)
Cocoa	High-flavanol chcolate vs. low-flavanol chocolate	40 g (first visit single dose); 20 g/day thereafter	Acute and 12-week, randomized, parallel	44 healthy pregnant women	HPLC-FLD (Targeted)	Plasma	• Epicatechin • Catechin • Caffeine • Theobromine • Theophylline	(157)
Cocoa	Cocoa in milk vs. whole milk	40 g (cocoa powder) in 250 mL (milk)	Acute, randomized, crossover	5 healthy volunteers	LC-MS/MS (Targeted)	Plasma, urine	• (-)-Epicatechin • (-)-Epicatechin glucuronide • (-)-Epicatechin sulfate	(158)
Cocoa	Alkalized cocoa powder in water	20.3 g (cocoa powder) in 400 mL water	Acute intervention	8 healthy volunteers	LC-MS/MS (Targeted)	Urine	• N-[4'-hydroxy-(E)-cinnamoyl]-L-aspartic acid • N-[4'-hydroxy-(E)-cinnamoyl]-L-glutamic acid • N-[4'-hydroxy-3'-methoxy-(E)-cinnamoyl]-L-tyrosine	(159)
Cocoa	Chocolate	80 g	Acute intervention	11 healthy volunteers	GC-MS, HPLC-ESI-MS/MS (Targeted)	Urine	• M-Hydroxyphenylpropionic acid • Ferulic acid • 3,4-dihydroxyphenylacetic acid • M-hydroxyphenylacetic acid • Vanillic acid • M-Hydroxybenzoic acid	(160)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cocoa	Cocoa in whole milk vs. cocoa in water	40 g (cocoa powder) in 250 mL (milk or water)	Acute, randomized, crossover	21 healthy volunteers	LC-MS/MS (Targeted)	Urine	• 3,4-dihydroxyphenylacetic acid • Phenylacetic acid • Protocatechuic acid • 4-hydroxybenzoic acid • 4-hydroxyhippuric acid • Hippuric acid • Vanillic acid • Caffeic acid • Ferulic acid	(161)
Cocoa	Cocoa beverage vs. whole milk	40 g (cocoa powder) in 250 mL water; 250 mL (milk)	Acute, randomized, crossover	21 healthy volunteers	LC-MS/MS (Targeted)	Urine	• (-)-Epicatechin glucuronide • (-)-Epicatechin sulfates	(162)
Cocoa	Chocolate (M&Ms semi-sweet chocolate mink baking bits) at 4 doses	0, 27, 53, 80 g	Acute, randomized, crossover	20 healthy volunteers	LC-Coulochem II coulometric detector (Targeted)	Plasma	• (-)-Epicatechin	(163)
Cocoa	Chocolate vs. cocoa	35 g cocoa powder in each	Acute crossover	5 healthy men	HPLC-MS (Targeted)	Plasma, urine	• (-)-Epicatechin and metabolites (glucuronides, sulfates, sulfoglucuronides, non-methylated, methylated)	(164)
Cocoa	Cocoa drink with low flavanols, medium flavanols, vs. high flavanols	18 g cocoa beverage mix in 250 mL water	Acute, randomized, double-blind, controlled, crossover	10 diabetic patients	HPLC-FLD (Targeted)	Plasma	• Total flavanols (epicatechin, catechin, and methylated, glucuronidated)	(165)
	Cocoa drink with high flavanols vs. low flavanols		30-day, randomized, double-blind, controlled, parallel	40 diabetic patients				
Cocoa	High-flavanol dark chocolate vs. low-flavanol dark chocolate	46 g	2-week, randomized, double-blind, placebo-controlled, parallel	21 healthy volunteers	HPLC-ECD (Targeted)	Plasma	• Epicatechin	(166)
Cocoa	Dark chocolate (85%) vs. milk chocolate (≤35%)	40 g	Acute, randomized, single-blind, crossover	20 patients with peripheral artery disease	HPLC-UV (Targeted)	Serum	• Epicatechin • Epicatechin-3-O-methylether • Epigallocatechin-3-gallate	(167)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cocoa	Chocolate vs. cola vs. caffeine capsules vs. theobromine capsules	82 g (chocolate); 800 mL (cola); 72 mg (caffeine capsules); 370 mg (theobromine capsule)	Acute crossover	7 healthy volunteers	HPLC-DAD (Targeted)	Plasma	• Methylxanthine • Caffeine • Paraxanthine • Theophylline • Theobromine	(168)
Cocoa	Cocoa drink vs. sugar	36 g cocoa powder	2-week, randomized, controlled, parallel	15 healthy men	HPLC-amperometric ECD (Targeted)	Plasma, urine	• Epicatechin	(169)
Cocoa	Nestle Noir dark chocolate low-dose vs. high-dose	40 g; 80 g	Acute crossover	8 healthy men	HPLC-DAD/FLD (Targeted)	Plasma	• Theobromine • Epicatechin	(170)
Cocoa	Dark chocolate vs. milk chocolate	200 g	3-day, randomized, controlled, parallel	58 healthy volunteers	Enzymatic method (oxalate)	Urine	• Oxalate	(171)
Cocoa	Dark chocolate (70%) vs. white chocolate (4% cocoa)	1 g/kg body weight	Acute crossover	10 healthy volunteers	Not reported	Plasma	• Catechin • Epicatechin and metabolites	(172)
Cocoa	Dark chocolate vs. theobromine sodium acetate oral solution	6 mg/kg body weight of theobromine (chocolate); 10 mg/kg body weight theobromine (oral solution)	7-day intervention (chocolate); acute intervention (oral solution)	12 healthy volunteers	HPLC-solit scintillator flow cell detector (Targeted)	Urine, serum	• Theobromine	(173)
Cocoa	Milk chocolate vs. chocolate powder vs. dark chocolate	40 g/day	7-day crossover	20 healthy volunteers	HPLC-PDA (Targeted)	Urine	• Theobromine	(174)
Cocoa	Non-alkalized cocoa beverage vs. alkalized cocoa beverage	0.6 g/kg body weight (alkalized powder); 0.8 g/kg body weight (non-alkalized powder) in 6 mL/kg body weight skimmed milk	Acute, randomized, double-blind, crossover	12 healthy volunteers	HPLC-CEAD (Targeted)	Plasma	• Epicatechin	(175)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Cocoa	Conventional cocoa beverage vs. flavanol-rich cocoa beverage	15 g (conventional); 25 g (flavanol rich) in 200 mL semi-skimmed milk	Acute, radomised, blind, crossover	13 healthy volunteers	HPLC-ESI-QTOF-MS (Targeted)	Plasma, urine	• Epicatechin metabolites (Epicatechin-3-glucuronide, 3-methoxy-glucuronide, 3-sulfate, methoxy-sulfate) • Phenyl-gamma-valerolactone derivatives (DHPV lactones, 5-(3-Hydroxyphenyl)-γ-valerolactone-4-glucuronide, 5-(4-Hydroxyphenyl)-γ-valerolactone-3-glucuronide, 5-(Hydroxyphenyl)-γ-valerolactone-sulfate, 5-Phenyl-γ-valerolactone-methoxy-glucuronide, 5-Phenyl-γ-valerolactone-methoxy-sulfate, 5-(3-Hydroxyphenyl)-γ-valerolactone, 5-Phenyl-γ-valerolactone-3-glucuronide, 5-Phenyl-γ-valerolactone-3-sulfate) • Phenylvaleric acid derivatives (4-Hydroxy-5-(3,4-dihydroxyphenyl)valeric acid, 4-Hydroxy-5-(hydroxyphenyl)valeric acid-glucuronide, 4-Hydroxy-5-(hydroxyphenyl)valeric acid-sulfate) • Other microbial metabolites (3,4-Dihydroxyphenylpropionic acid, 3-Methoxy-4-hydroxyphenylpropionic acid 3-Hydroxyphenylpropionic acid 3,4-Dihydroxyphenylacetic acid, 3-Methoxy-4-hydroxyphenylacetic acid 3-Hydroxyphenylacetic acid, Ferulic acid Isoferulic acid, 3,4-Dihydroxybenzoic acid, 4-Hydroxyhippuric acid, 3-Hydroxyhippuric acid, Hydroxybenzoic acid)	(176)
Cocoa	Milk chocolate vs. hazelnut and cocoa spread	60 g	Acute, randomized, single-blind, crossover	20 healthy smokers	Folin-Ciocalteau method (polyphenols) ; GC-MS (vitamin E) (Targeted)	Plasma (polyphenols); serum (vitamin E)	• Total polyphenols • Vitamin E	(177)
Cocoa	Dark chocolate (90%)	50 g	Acute intervention	18 healthy men	U(H)PLC-ESI-MS/MS (Targeted)	Plasma	• Epicatechin and metabolites (glucuronides, glucuronide-sulfate, sulfates, methyl-epicatechin sulfates) • Phenyl-gamma-valerolactones (5-(3-Hydroxyphenyl)-g-valerolactone-4-glucuronide, 5-Phenyl-g-valerolactone-glucuronide-sulfate, 5-(4-Hydroxyphenyl)-g-valerolactone-3-glucuronide, 5-Phenyl-g-valerolactone-3-glucuronide, 5-(Hydroxyphenyl)-g-valerolactone-sulfate isomers, 5-Phenyl-g-valerolactone-sulfate-methoxy, 5-Phenyl-g-valerolactone-3-sulfate)	(178)
Cocoa	Cocoa-enriched dark chocolate (70%) vs. cocoa-depleted control chocolate	561 kcal	Acute, single-blind, randomized, crossover	16 healthy male cyclists	RP-HPLC-UV (Targeted)	Plasma	• Epicatechin • Theobromine	(179)
Coffee	Coffee consumption	0, 1, 2-4, 5-8, >8 cups of coffee/day (from questionnaire)	Association study	3503 patients undergoing elective coronary angiography	LC-MS/MS (Targeted)	Plasma	• Trigonelline • Nicotinamide • N-methylnicotinamide	(180)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
				from the WECAC cohort				
Coffee	Instant coffee (Nescafe Gold Blend)	3.4 g powder in 200 mL water	Acute intervention	5 volunteers with an ileostomy	HPLC-PDA-MS/MS (Targeted)	Ileal effluent, urine	• Total chlorogenic acid and metabolites • 3-, 4-, and 5-O-caffeoylquinic acids • 3-, 4-, and 5-O-caffeoylquinic acid sulfates • 3- and 4--O-caffeoylquinic acid glucuronides • 3-, 4-, and 5-O-feruloylquinic acids • 3-, 4-, and 5-O-feruloylquinic acid sulfate • 3- and 4-O-feruloylquinic acid glucuronides • 3- and 4-O-caffeoylquinic acid lactones • 3- and 4-O-caffeoylquinic glucuronides and sulfates • 4- and 5-O-p-coumaroylquinic acid • 3,4-, 3,5-, and 4,5-O-di-caffeoylquinic acid • Caffeic acid • Caffeic acid 4- and 3-O-sulfates • Ferulic acid • Ferulic acid-4-O-sulfate • Feruloylglycine • Dihydroferulic acid-4-O-sulfate • Isoferulic acid 3-O-sulfate and glucuronide • Dihydrocaffeic acid-3-O-sulfate	(181)
Coffee	Instant coffee	200 mL	Acute intervention	11 healthy volunteers	HPLC-PDA-MS/MS (Targeted)	Plasma, urine	• Dihydrocaffeic acid 3'-sulfate • Dihydrocaffeic acid-3'-O-glucuronide • Caffeic acid 4'-sulfate • Dihydroferulic acid 4'-sulfate • Caffeic acid 3'-sulfate • Dihydroferulic acid 4'-O-glucuronide • Ferulic acid 4'-sulfate • Isoferulic acid 3'-sulfate • Dihydroisoferulic acid 3'-O-glucuronide • Isoferulic acid 3'-O-glucuronide	(182)
Coffee	Coffee vs. tea vs. Cola soft drink	At least 1 cup of coffee or tea, 500 mL soft drink	Acute intervention	146 healthy volunters	HPLC-UV (Targeted)	Urine	• 5-acetylamino-6-formylamino-3-methyluracil • 1-methylxanthine	(183)
Coffee	Low-polyphenol coffee vs. high-polyphenol coffee vs. caffeine in hot water	3.6 g ground coffee to 50 mL water; 110 mg caffeine	Acute, randomized, controlled, crossover	15 healthy men	U(H)PLC-ESI-MS/MS (Targeted)	Plasma	• Total chlorogenic acid metabolites • 3-caffeoylquinic acid • 4-caffeoylquinic acid • Caffeic-4-O-sulfate • 3-feroylquinic acid • 4-feroylquinic acid	(184)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• 5-feroylquinic acid • Ferulic acid • Isoferulic acid • Methylferulic acid • Ferulic-4-O-glucuronide • Isoferulic-3-O-glucuronide • Ferulic-4-O-sulfate • Isoferulic-3-O-sulfate	
Coffee	Caffeinated coffee vs. decaffeinated coffee vs. combination	2 cups (caffeinated); 2 cups (decaffeinated); 1 cup (caffeinated) + 1 cup (decaffeinated)	Acute, randomized, crossover	17 men with coronary artery disease performing a treadmill test	HPLC (detector not specified) (Targeted)	Serum	• Caffeine	(185)
Coffee	Coffee (mostly instant coffee) and tea	Number of cups of coffee and tea consumption (FFQ for usual consumers; 24-h recall for current consumers)	Association study	111 free-living volunteers (usual consumers); 344 healthy volunteers (current consumers)	GC-MS (Targeted)	Urine	• Isoferulic acid	(186)
Coffee	Filtered coffee	0 (refrain from consumption), 4 cups/day, 8 cups/day (150 mL/cup)	1-month, crossover	47 habitual coffee drinkers	HPLC-MS, GC-MS (Targeted)	Serum, plasma	• Caffeine • Paraxanthine • Theobromine • Theophylline • Caffeic acid • Dihydrocaffeic acid • Ferulic acid • Isoferulic acid • Dihydroferulic acid • Dihydroisoferulic acid • 3-(3-Hydroxyphenyl)propanoic acid • 3-(3,4-Dimethoxyphenyl)propanoic acid • 3,4-Dimethylcaffeic acid • 3-Coumaric acid	(187)
Coffee	Coffee	Cups of coffee consumption (1-2, 3-7, >8) (from survey)	Association study	284 healthy men	LC-ESI-MS/MS (Targeted 363 metabolites)	Serum	• Sphingomyelin SM(OH,COOH) • Sphingomyelin SM(OH) • Long- and medium-chain acylcarnitines	(188)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Coffee	Coffee	Non-coffee consumers (0 mL/day), low-coffee consumers (≤100 mL/day), high-coffee consumers (>100 mL/day) (from 24-h recalls and FFQ)	Association study	169 healthy volunteers	FIA-MS/MS, HPLC-MS/MS (Targeted)	Plasma	• 3-, 4-, 5-Caffeoylquinic acid • 5-Feruloylquinic acid • 4-Ethylguaiacol • 4-Vinylguaiacol • Catechol • Pyrogallol • LysoPC C16:0/16:1 • LysoPC C18:0/18:1	(189)
Coffee	Coffee and tea	Coffee and tea consumption (from FFQ and 3-day food record)	Association study	57 healthy women	HPLC-MS/MS (Targeted)	Plasma, urine	• Caffeic acid • Chlorogenic acid	(190)
Coffee	Coffee	Preparation: 48 g coffee powder in 900 mL water; 350 mL consumed	Acute intervention	13 healthy volunteers	HILIC-MS/MS (Targeted)	Plasma	• Trigonelline • N-methylpyridinium	(191)
Coffee	Coffee vs. tea vs. water	Dose not specified	Acute intervention	13 healthy volunteers	CE-ESI-TOF-MS, HPLC-MS/MS (Targeted)	Urine	• Chlorogenic acid • Caffeic acid • Coumaric acid	(192)
Coffee	Coffee	Preparation: 40 g coffee powder in 250 mL water' 190 mL consumed	Acute intervention	6 healthy volunteers	HPLC-UV, LC-MS (Targeted)	Plasma, urine	• 3-, 4-, and 5-Caffeoylquinic acid (plasma) • 3,4-, 3,5-, and 4,5-Dicaffeoylquinic acid (plasma) • Feryloylquinic acid (plasma, only 1 volunteer) • Dihydrocaffeic acid (urine) • Gallic acid (urine) • Isoferulic acid (urine) • Ferulic acid (urine) • Vanillic acid (urine) • Caffeic acid (urine) • 5-caffeoylquinic acid (urine) • Sinapic acid (urine) • P-hydroxybenzoic acid (urine) • P-coumaric acid (urine)	(193)
Coffee	Instant coffee with low chlorogenic acids vs. medium chlorogenic acids vs. high	3.4g coffee powder in 200 mL water	Acute, randomized, double-blind, controlled, crossover	11 healthy volunteers	HPLC-PDA-MS/MS (Targeted)	Plasma, urine	• 3-Caffeoylquinic acid-O-sulfate • Dihydrocaffeic acid-3-O-sulfate • Dihydrocaffeic acid-3-O-glucuronide • 4-caffeoylquinic acid-O-sulfate • Caffeic acid-4-O-sulfate • Dihydroferulic acid-4-O-sulfate • Caffeic acid-3-O-sulfate	(194)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
	chlorogenic acids						• Dihydrocaffeic acid • Dihydroferulic acid-4-O-glucuronide • Ferulic acid-4-O-sulfate • 5-caffeoylquinic acid • 3-feruloylquinic acid • Isoferulic acid-3-O-sulfate • Dihydroisoferulic acid-3-O-glucuronide • Isoferulic acid-3-O-glucuronide • Feruloylglycine • 3-Caffeoylquinic acid lactone-O-sulfate • 4-Caffeoylquinic acid lactone-O-sulfate • 4-Feruloylquinic acid • Dihydroferulic acid • 5-Feruloylquinic acid	
Coffee	Coffee at 3 doses	2, 4, and 8 g coffee powder in 400 mL water	Acute, randomized, crossover	10 healthy volunteers	LC-ESI-MS/MS (Targeted)	Plasma	• 3-Feruloylquinic acid • 4-Feruloylquinic acid • 5-Feruloylquinic acid • 3-Caffeoylquinic acid • 4-Caffeoylquinic acid • 5-Caffeoylquinic acid • Ferulic acid • Caffeic acid • Isoferulic acid • Dihydroferulic acid • Dihydrocaffeic acid	(195)
Coffee	Coffee vs. no coffee	1 cup (acute); daily consumption of coffee	Acute intervention	Healthy volunteers (number not specified)	HPLC-UV (Targeted)	Urine	• N-methyl-2-pyridone-5-carboxamide • N-methyl-4-pyridone-5-carboxamide	(196)
Coffee	Hot coffee consumed slowly vs. cold coffee consumed slowly vs. cold coffee consumed fast vs. sugar-free energy drink consumed fast vs. sugar-free energy drink consumed slowly	4.1 g coffee powder in 450 mL water; 450 mL energy drink	Acute, randomized, crossover	24 healthy volunteers	LC-MS (Targeted)	Plasma	• Caffeine	(197)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Coffee	Coffee vs. soy beverage vs. coffee + soy beverage	4 g coffee powder and/or 20 g soymilk powder in 200 mL water	Acute, randomized, crossover	6 healthy volunteers	HPLC-DAD, LC-MS (Targeted)	Urine	• 3-Caffeoylquinic acid • 4-Caffeoylquinic acid • 5-Caffeoylquinic acid • 3,4-Dicaffeoylquinic acid • 3,5-Dicaffeoylquinic acid • 4,5-Dicaffeoylquinic acid • Ferulic acid • Caffeic acid • Isoferulic acid • Dihydrocaffeic acid • Gallic acid • Vanillic acid • Benzoic acid • P-Hydroxybenzoic acid • Siringic acid • Sinapic acid • 3,4-Dihydroxyphenilacetic acid • Hippuric acid • Trans-3-hydroxycinnamic acid • 3-(4-Hydroxyphenyl) propanoic acid • 2,4-Dihydroxybenzoic acid	(198)
Coffee	Instant coffee	1 cup containing 4 mg caffeine/kg body weight	Acute intervention	6 healthy volunteers	HPLC-MS/MS (Targeted)	Urine	• AAMU/AFMU • 1-Methyluric acid • 1-Methylxanthine • 1,3-Dimethyluric acid • 1,7-Dimethyluric acid • 1,3,7-Trimethyluric acid • 3,7-Dimethylxanthine • Paraxanthine • 1,3-Dimethylxanthine • 1,3,7-Trimethylxanthine	(199)
Coffee	Instant coffee	400 mL	Acute intervention	8 healthy volunteers	HPLC-MS/MS (Targeted)	Plasma	• 3,4-Dimethoxycinnamic acid	(200)
Coffee	Instant coffee	4 g in 400 mL water	Acute intervention	Healthy volunteers (number not specified)	LC-MS/MS (Targeted)	Plasma	• Caffeic acid • Dihydrocaffeic acid • Ferulic acid • Dihydroferulic acid • Isoferulic acid	(201)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Coffee	Instant coffee	3.4 g in 200 mL	Acute intervention	11 healthy volunteers	HPLC-PDA-MS/MS (Targeted)	Plasma, urine	• 3-O-Caffeoylquinic acid-O-sulfate • DIhydrocaffeic acid-3-O-sulfate • DIhydrocaffeic acid-3-O-glucuronide • 4-O-Caffeoylquinic acid-O-sulfate • Caffeic acid-4-O-sulfate • Dihydroferulic acid-4-O-sulfate • Caffeic acid-3-O-sulfate • Dihydrocaffeic acid • Dihydroferulic acid-4-O-glucuronide • Ferulic acid-4-O-sulfate • 5-O-Caffeoylquinic acid • 3-O-Feruloylquinic acid • Isoferulic acid-3-O-sulfate • Dihydro(*iso*)ferulic acid-3-O-glucuronide • Isoferulic acid-3-O-glucuronide • Feruloylglycine • 3-O-Caffeoylquinic acid lactone-O-sulfate • 4-O-Caffeoylquinic acid lactone-O-sulfate • 4-O-Feruloylquinic acid • Dihydroferulic acid • 5-O-Feruloylquinic acid	(202)
Coffee	Instant coffee in water vs. instant coffee in milk vs. water	4 g in 200 mL water or milk	Acute, randomized, crossover	5 healthy volunteers	HPLC-DAD, LC-MS (Targeted)	Urine	• Hippuric acid • 3,4-Dihydroxyphenylacetic acid • Dihydrocaffeic acid • Vanillic acid • Gallic acid • Isoferulic acid • 4-Hydroxybenzoic acid • 2,4-Hydroxybenzoic acid • Trans-3-hydroxycinnamic acid • P-Coumaric acid • Syringic acid • Sinapinic acid	(203)
Coffee	Freshly brewed coffee	30 g in 500 mL water	Acute intervention	10 healthy volunteers	HPLC-ESI-MS/MS (Targeted)	Urine	• Nicotinic acid • Nicotinamide • N-methylnicotinamide • N-methyl-2-pyridone-5-carboxamide • Nicotinuric acid	(204)
Coffee	Green-roasted coffee	3.5 gin 250 mL water	Acute intervention	12 healthy volunteers	HPLC-DAD, LC-MS-QTOF (Targeted)	Plasma, urine	• Caffeine • Paraxanthine • Theobromine • Theophylline • 1-Methylxanthine	(205)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• 3-Methylxanthine • 7-Methylxanthine • 1-Methyluric acid • 1,3-Methyluric acid • 1,7-Methyluric acid • 1,3,7-Methyluric acid	
Coffee	Instant coffee	8 g in 400 mL water	Acute intervention	10 healthy volunteers	LC-ESI-MS/MS (Targeted 56 phenolic compounds)	Plasma	• Total phenolic acids, methyls, glucuronides, sulfates, and lactones (ferulic acid, caffeic acid, and all metabolites thereof)	(206)
Coffee	Instant coffee	4 g in 400 mL water	Acute intervention	9 healthy volunteers	ESI-LC-ESI-MS/MS (Targeted)	Plasma	• Ferulic acid • Caffeic acid • Isoferulic acid • Dihydrocaffeic acid • Dihydroferulic acid	(207)
Coffee	Instant coffee vs. green tea	4 g in 400 mL water; 1.25% tea infusion	Acute crossover	9 healthy volunteers	ESI-LC-ESI-MS/MS (Targeted)	Plasma	• Ferulic acid • Caffeic acid • Isoferulic acid • Dihydrocaffeic acid • Dihydroferulic acid	(208)
Coffee	Instant coffee vs. instant coffee in whole milk vs. instant coffee with sugar and non-dairy creamer vs. no coffee	4 g in 400 mL water or 360 mL water + 40 mL whole milk or 30.5 g premixed instant coffee with sugar	Acute, randomized, crossover	9 healthy volunteers	ESI-LC-ESI-MS/MS (Targeted)	Plasma	• Ferulic acid • Caffeic acid • Isoferulic acid	(209)
Coffee	Coffee	0, 1, 2, or 3 cups/day	Cross-sectional	15 healthy men	LC-MS/MS (Targeted)	Urine	• Hippuric acid	(210)
Coffee	Nescafe Green Blend coffee	3.5 g in 240 mL water	Acute intervention	12 healthy volunteers	LC-MS-QTOF (Targeted)	Plasma, urine	• Caffeic acid and metabolites • Ferulic acid and metabolites • Coumaric acid and metabolites • Dimethoxycinnamic acid and metabolites • Lactone derivatives • Phenolic acids	(211)
Coffee	Coffee	Caffeinated coffee, other caffeinated drinks, decaffeinated coffee consumption	Association study	598 volunteers rom the SKIPOGH cohort	HPLC-MS/MS (Targeted)	Urine	• Caffeine • Paraxanthine • Theophylline	(212)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
		(never, 1-4X/month, 1-4X/week, 5X/week, ≥1X/day) (from FFQ)						
Coffee	Instant coffee	3.4 g in 200 mL water	Acute intervention	11 healthy volunteers	HPLC-PDA-MS (Targeted)	Urine	• Caffeic acid-3-O-sulfate • Caffeic acid-3-O-sulfate • Ferulic acid-4-O-sulfate • Isoferulic acid-3-O-glucuronide • Isoferulic acid-3-O-sulfate • Dihydrocaffeic acid-3-O-glucuronide • Dihydrocaffeic acid-3-O-sulfate • Dihydroferulic acid • Dihydroferulic acid-4-O-glucuronide • Dihydroferulic acid-4-O-sulfate	(213)
Coffee	Coffee enema vs. ready-to-drink coffee beverage	500 mL (coffee enema); 180 mL (RTD coffee)	Acute crossover	11 healthy men	HPLC-UV (Targeted)	Plasma	• Caffeine	(214)
Coffee	Decaffeinated coffee with high chlorogenic acids vs. medium vs. low	Coffee dose not reported; 4525, 2219, and 1052 umol chlorogenic acids in high, medium, and low doses, respectively	Acute, randomized, double-blind, crossover	5 healthy womn	HPLC-DAD-ESI-MS, ESI-MS/MS (Targeted)	Plasma, ileal effluent, urine	• Chlorogenic acid metabolites (caffeoylquinic acids, feruloylquinic acids, caffeic acids, dihydrocaffeic acids, ferulic acids, isoferulic acids, dihydroferulic acids)	(215)
Coffee	Instant coffee	400 mL (1% w/v)	Acute intervention	10 healthy volunteers	LC-MS (Targeted)	Plasma	• Caffeic acid • Dihydrocaffeic acid • Ferulic acid • Isoferulic acid • Dihydroferulic acid • Dimethoxycinnamic acid • Dimethoxydihydrocinnamic acid	(216)
Coffee	Coffee	2 cups	Acute intervention	14 healthy volunteers	2D-HR-GC-MS (Targeted)	Urine	• 4-Ethylguaiacol • 4-Vinylguaiacol • (E)-Beta-damascenone • Dimethyl trisulfide • Furfuryl alcohol • Guaiacol • Indole • Methional • Oct-1-en-3-one	(217)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
							• Skatole • Vanillin	
Coffee	Mocha coffee vs. caffeinated soft drink vs. low-dose caffeine in aqeuous solution vs. high-dose caffeine in aqueous solution	190 mL (soft drink), 50 mL (mocha), 70 mL (aqueous solution	Acute, randomized, crossover	4 healthy men	HPLC (detector not specified) (Targeted)	Plasma	• Caffeine • Theophylline • Paraxanthine • Theobromine • 1,3-Dimethylxanthine • 1,7-Dimethylxanthine • 3,7-Dimethylxanthine	(218)
Coffee	Coffee (brewed or canned)	150 mL (brewed coffee) or 187 mL (canned coffee)	Acute intervention	10 healthy volunteers	FOX-I method (Targeted)	Urine	• Hydrogen peroxide	(219)
Coffee	Instant coffee	2.5 g instant coffee in 200 mL water	Acute intervention	10 healthy volunteers	FOX assay (Targeted)	Urine	• Hydrogen peroxide	(220)
Coffee	Instant coffee	2 cups, each with 4 g instant coffee powder in 250 mL water	Acute intervention	5 healthy men	HPLC-UV (Targeted)	Urine	• Caffeic acid • Ferulic acid • Isoferulic acid • Dihydroferulic acid • 3-4-Hydroxy-3-methoxyphenyl-propanoic acid • Vanillic acid • Hippuric acid • 3-Hydroxyhippuric acid	(221)
Coffee	High roasted coffee vs. low roasted coffee vs. unroasted coffee vs. in vitro hydrolyzed unroasted coffee	3.4 to 4.5 g of instant coffee in 200 mL water	Acute, randomized, double-blind, crossover	12 healthy volunteers	LC-ESI-MS/MS (Targeted)	Plasma	• Dihydroferulic acid • Caffeic acid-3-O-sulfate • Isoferulic-3-O-glucuronide • 5-4-Dihydro-m-coumaric acid	(222)
Coffee	Decaffeinated coffee vs. regular coffee vs. stronger coffee vs. hot water vs. no intervention	17.5 to 25 g of coffee in 300 mL water	Acute, randomized, single-blind, crossover	8 healthy volunteers	HPLC (detector not specified) (Targeted)	Plasma	• Caffeine	(223)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Coffee	Coffee	4X7.5 g coffee pads and 500 mL water	Acute intervention	10 healthy volunteers	HPLC-ESI-MS/MS (Targeted)	Urine	• Pyrazine-2-carboxylic acid • 5-Hydroxypyrazine-2-carboxylic acid • 5-Methylpyrazine-2-carboxylic acid • 6-Methylpyrazine-2-carboxylic acid	(224)
Coffee	Coffee drinkers vs. non-coffee drinkers	350 mL coffee or water	Acute, parallel	6 healthy volunteers	UPLC-HDMS (Untargeted)	Urine	• Trigonelline • N-methylpyridinium • Dimethylxanthines • Monomethylxanthines • 1,3-Dimethyluric acid • 1,7-Dimethyluric acid • Ferulic acid conjugates	(225)
	Caffeinated coffee	48 g coffee powder in 900 mL water	Acute intervention	13 healthy volunteers	UPLC-MS/MS (Targeted)	Plasma	• 5-O-Caffeoyl quinic acid • Ferulic acid • Iso-ferulic acid • Feruloylsulfate • Iso-feruloylsulfate • Feruloylglucuronide • Feruloylglycine • Dihydroferulic acid • Dihydroferuloylsulfate • Dihydroferuloylglucuronide • Dihydrocaffeoylsulfate (sum of isomers) • Catecholsulfate • Catecholglucuronide • Guaiacolsulfate • Guaiacolglucuronide • Trigonelline • N-methylpyridinium • N-methylnicotinamide • N-methyl-4-pyridone-5-carboxamide • N-methyl-2-pyridone-5-carboxamide • Caffeine • Theophylline • Paraxanthine • Theobromine • 3-Methylxanthine • 7-Methylxanthine • 1,7-Dimethyluric acid	

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Coffee, cocoa	Green tea vs. grape-skin extract vs. cocoa beverage vs. instant coffee coffee vs. grape fruit juice vs. orange juice vs. hot water	4 g (instant coffee), 10 g (cocoa powder) in 200 mL water	Acute, randomized, crossover	9 healthy volunteers	HPLC-ESI-MS/MS (Targeted)	Urine	• Chlorogenic acid • Caffeic acid • M-coumaric acid • 4-O-methylgallic acid • Epicatechin • Naringenin • Enterodiol • Enterolactone	(226)
Coffee, wine	Coffee, chocolate, wine, dark bread, and other conventional foods	Continuous intake of various foods (from 2-day dietary record)	Association study	53 volunteers from the SU.VI.MAX cohort	HPLC-ESI-MS/MS (Targeted)	Urine	• Chlorogenic acid • Caffeic acid • Gallic acid • 4-O-methylgallic acid • Enterolactone • Enterodiol	(227)
Coffee, wine (polyphenol-rich foods)	Citrus fruits, apple and pear, olives, coffee tea, all wine, red wine	Continuous intake of citrus fruits, apple and pear, olives, coffee tea, all wine, red wine (from 24-h recalls and dietary questionnaire)	Association study	475 volunteers from the EPIC cohort	U(H)PLC-ESI-MS/MS (Targeted 34 polyphenols)	Urine	• Protocatechuic acid, 3,4-dihydroxyphenylpropionic acid, ferulic acid, and caffeic acid highly associated with coffee intake (also: gallic acid, apigenin, quercetin, homovanillic acid, protocatechuic acid, m-coumaric acid, hydroxytyrosol, and daidzein, based on ranked method) • Hydroxytyrosol, tyrosol, resveratrol, gallic acid, and gallic acid ethyl ester highly associated with wine/red wine intake (also: homovanillic acid, 3-hydroxybenzoic acid, naringenin, 3,4-dihydroxyphenylpropanoic acid, 3,4-dihydroxyphenylacetic acid, p-coumaric acid, enterolactone, and catechin, based on ranked method)	(228)
Coffee, wine, cocoa (polyphenol-rich foods)	Coffee, tea, red wine, citrus fruit, apples and pears, and chocolate products	Continuous intake of coffee, tea, red wine, citrus fruit, apples and pears, and chocolate products (from 24-h recall and FFQ)	Association study	481 volunteers from the EPIC cohort	U(H)PLC-QTOF-MS (Targeted)	Urine	• Dihydroferulic acid sulfate, guaiacol glucuronide, feruloylquinic acid, ferulic acid sulfate, feruloylquinic acid glucuronide, 3-O-caffeoylquinic acid, p-coumaric acid sulfate, caffeic acid sulfate, ferulic acid glucuronide, hydroxyhippuric acid, dihydrocaffeic acid sulfate, m-coumaric acid sulfate, dihydroferulic acid glucuronide, p-hydroxyphenyllactic acid, guaiacol sulfate, ethylcatechol glucuronide associated with coffee intake • M-coumaric acid, gallic acid ethyl ester sulfate, hydroxytyrosol sulfate, dihydroresveratrol glucuronide, syringic acid sulfate, methylgallic acid sulfate, 4-O-methylgallic acid associated with red wine intake • Methyl(epi)catechin sulfate, 4-hydroxy-(3'4'-dihydroxyphenyl)valeric acid sulfate, dihydroxyphenyl-gamma-valerolactone glucuronide, vanillic acid sulfate associated with chocolate intake	(229)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Coffee, bread, cheese (general diet)	Wholegrain bread, non-wholegrain bread, lowfat cheese, highfat cheese, regular coffee, decaffeinated coffee, and other (non-fermented) food groups	Continuous intake of 45 different food groups (from FFQ)	Association study	2380 volunteers from the EPIC-Potsdam cohort	FIA-MS/MS (Targeted 127 metabolites, including acylcarnitines, amino acids, diacyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines, lyso-phosphatidylcholines, sphingomyelins, and hexoses)	Serum	• Amino acids associated with all dairy • Hexoses with non-wholegrain bread • Acyl-alkyl-phosphatidylcholines and lyso-phosphatidylcholines with high-fat dairy • Sphingomyelins with coffee	(230)
Coffee, wine	Coffee, tea, wine, cereal, fruit, vegetable, other food intake	Continuous intake of coffee, tea, wine, cereal, fruit, vegetable, other foods (from 7-day weighted diet records); from FFQ	Association study	61 volunteers, most with CV risk; 2672 volunteers from the NORKOST 2 cohort	HPLC-UV (Targeted)	Plasma	• Zeaxanthin • Beta-carotene • Alpha-carotene	(231)
Coffee, cocoa	Espresso coffee vs. espresso coffee + cocoa-based products containing coffee	3 cups espresso vs. 1 cup espresso + 2 cups cocoa-based products containing coffee	1-month, randomized, crossover	21 healthy volunteers	UHPLC-ESI-MS (Targeted)	Plasma, urine	• Trigonelline (coffee) • N-methylpyridinium (coffee) • N-methylnicotinamide (coffee) • N-methyl-4-pyridone-5-carboxamide (coffee)	(232)
Vinegar	Vinegar capsules vs. vinegar drink vs. non-carbonated mineral water	9 capsules (750 mg acetic acid); 100 mL vinegar (750 mg acetic acid); 150 mL water	Acute, randomized, controlled, crossover	30 healthy volunteers	GC-2010 (Targeted)	Serum	• Acetate	(233)
Vinegar	Red wine vinegar vs. apple cider vinegar tablet	4 tablespoons/day (3.6 g acetic acid/day); 2 tablets/day (0.045 g acetic acid/day)	8-week, randomized, controlled, parallel	45 healthy volunteers with high waist circumference and at increased	GC-MS, LC-MS/MS (Targeted)	Plasma	• Amino valerate • Indole-3-acetic acid • dTMP	(234)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
				risk for metabolic complications				
Sauerkraut	Sauerkraut	5-6 g/kg body weight	Acute intervention	4 volunteers	LC-MS (Targeted)	Plasma, urine	• D-phenyllactic acid	(235)
Cider	Thatchers Redstreak apple cider	500 mL	Acute intervention	9 healthy and 5 ileostomy volunteers	HPLC-PDA-MS (Targeted)	Plasma, urine, ileal fluid	• Phloretin-2'-glucuronide • Phloretin-O-glucuronide-O-sulfate • Phoretin-O-sulfate	(236)
Fermented orange juice	Fermented orange juice (fermented using yeast: *Pichia kluyveri* var. *kluyveri*) vs. unfermented orange juice	500 mL	Acute, randomized, controlled, crossover	7 healthy volunteers	HPLC-DAD (Targeted)	Plasma	• Beta-cryptoxanthin • Lutein	(237)
Fermented ginseng	Fermented vs. non-fermented ginseng	3 g	Open-label, randomized, single-dose, crossover	24 healthy volunteers	LC-MS/MS (Targeted)	Plasma	• Ginsenoside metabolite IH-901 (20-O-beta-D-glucopyranosyl-20(S)-protopanaxodiol)	(238)
Fermented Red Beet Juice	Fermented red beet juice	200 mL/60 kg body weight	6-week, uncontrolled, intervention	24 healthy volunteers	Micro-HPLC-MS/MS (Targeted)	Plasma, urine	• Betalain and derivatives (isobetanin, isobetanidin, 17-decarboxy-betanin, 17-decarboxy-isobetanin, 17-decarboxy-neobetanin, neobetanin, 2,17-bidecarboxyneobetanin, 2,15,17-tiidecarboxyneobetanin, 2,17-bidecarboxyneobetanin, 2,15,17-tiidecarboxybetanin, 6'-O-feruloyl-betanin/isobetanin, , 2,15,17-tiidecarboxy-2,3-dehydro-neobetanin)	(239)
Salgam, boza, kimiz, kefir	Salgam, boza, kimiz, kefir	300 mL	Acute, crossover	12 healthy volunteers	HS-GC-FID (blood ethanol); LC-MS/MS (urine ethyl glucuronide and sulfate) (Targeted)	Whole blood, urine	• No change in blood alcohol levels or ethanol metabolites in urine	(240)
Fermented red cabbage	Fermented red cabbage vs. fresh red cabbage	240 g	Acute, randomized, controlled, crossover	13 healthy volunteers	HPLC-MS/MS (Targeted)	Plasma, urine	• Cyanidin derivatives	(241)

Table S4. Summary of targeted studies presenting candidate FIBs for fermented foods

Fermented Food	All interventions	Dose	Study Design	*n*	Analytical Method	Biosample	Candidate FIBs[a]	Reference
Fermented rooibos tea	Fermented vs. unfermented rooibos tea	500 mL	Acute, randomized, controlled, crossover	10 healthy volunteers	HPLC-MS (Targeted)	Plasma, urine	• C-linked dihydrochalcone and flavanone glucosides (O-methyl, sulfate, glucuronide metabolites of aspalathin and eriodictyol-O-sulfate	(242)
Pu-erh tea	Pu-erh tea	200 mL (containing 10 g of tea powder)	Acute and 2-week, randomized, controlled	20 healthy volunteers	U(H)PLC-QTOF-MS (Targeted)	Urine	• Inositol • Myristic acid • 5-Hydroxytryptophan • 4-Methyloxyphenylacetic acid • Pyroglutamic acid	(243)

AR, alkenylresorcinol; CE, capillary electrophoresis; CEAD, coulometric electrode array detector; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DAD, diode array detector; DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-1-propanoic acid); DHPPTA, 5-(3,5-dihydroxyphenyl) pentanoic acid; DHPV, 5-(3,4-dihydroxyphenyl)-γ-valerolactone; EAD, enzyme activity/affinity detector; ECD, electrochemical detector; ELISA, enzyme-linked immunosorbent assay; EPA, eicosapentaenoic acid; ESI, electrospray ionization; FFQ, food frequency questionnaire; FIA, flow injection analysis; FIB, food intake biomarker; FID, flame ionization detector; FLD, fluorescence/fluorometric detector; FOX, ferrous ion oxidation xylenol orange; GC, gas chromatography; GLC, gas-liquid chromatography; HDMS, high definition mass spectrometry; HILIC, hydrophilic interaction liquid chromatography; HPLC, high-performance liquid chromatography; HR, high resolution; HS, headspace; LC, liquid chromatography; LDL, low-density lipoprotein; MHPV, 3′-methoxy-4′-hydroxyphenylvalerolactone; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MUFA, monounsaturated fatty acid; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PDA, photometric diode array; PUFA, polyunsaturated fatty acid; QTOF, quadrupole time-of-flight; RP, reverse phase; SCFA, short chain fatty acid; SFA, saturated fatty acid; TOF, time-of-flight; U(H)PLC, ultra-high performance liquid chromatography; UV, ultraviolet; VIS, visible.

[a] Candidate FIBs that are significantly increased compared to control or baseline in each study are reported.

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CHAPTER 3

Prevalence of fermented foods in the Dutch adult diet and validation of a food frequency questionnaire for estimating their intake in the NQplus cohort

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Abstract

Background: Humans have a long history of consuming fermented foods. However, their prevalence in human diets remains largely undetermined, and there is a lack of validated dietary assessment tools assessing the intake of different fermented products. This study aimed to identify fermented foods consumed in The Netherlands and determine the relative validity of a food frequency questionnaire (FFQ) compared to multiple 24-h recalls for estimating their intake.

Methods: The validation population consisted of 809 participants (53.1±11.9 years) from a Dutch observational cohort (NQplus) who completed a FFQ and multiple 24-h recalls. Fermented foods from the FFQ and recalls were identified and aggregated into conventional food groups. Percent difference in mean intakes, quintile cross-classification, Spearman's correlations, and Bland-Altman analyses were used to evaluate the agreement between the two dietary assessment methods.

Results: Approximately 16-18% of foods consumed by this population were fermented, and a further 9-14% were dishes containing a fermented ingredient. Fermented foods with the highest consumption included coffee (~453g/day;~0.5% of daily energy intake), yoghurts (~88g/day;~2.2%), beer (~84g/day;~1.7%), wholegrain bread (~81g/day;~9.4%), wine (~65g/day;~2.7%), and cheese (~32g/day;~5.0%). Mean percent difference between the FFQ and recalls was small for fermented beverages (coffee), breads (brown, white, wholegrain, rye), and fermented dairy (cheeses) (0.3-2.8%), but large for buttermilk and quark (≥53%). All fermented food groups had >50% of participants classified into the same or adjacent quintile of intake (58%-buttermilk to 89%-fermented beverages). Strong Spearman's correlations (crude/energy-adjusted $r_s \geq 0.50$) were obtained for fermented beverages (coffee, beer, wine), cereals/grains (wholegrain bread), and dairy (yoghurts). For 'other bread', quark, and buttermilk, correlations were low ($r_s < 0.20$). Bland-Altman analyses revealed good agreement for fermented beverages (coffee, beer), breads (brown, wholegrain, rye, other), pastries, chocolate, and fermented dairy (cheeses) (mean difference: 0.1-9.3).

Conclusions: Fermented food groups with acceptable or good validity across all measures included commonly consumed foods in The Netherlands: fermented beverages (coffee), wholegrain and rye bread, and fermented dairy (cheeses). However, for less frequently consumed foods, such as quark and buttermilk, the levels of agreement were poor and estimates of intake should be interpreted with caution. This report provides the basis for developing a FFQ specific for fermented foods.

Background

Fermented foods are foods or beverages in which microorganisms have been intentionally added or used to enzymatically transform food components (1). They comprise a large, pervasive group of foods in the Western diet, including cheese, yoghurt, buttermilk, coffee, beer, wine, bread, sauerkraut, dried sausages, and chocolate. The fermentation process not only improves the shelf-life and organoleptic qualities of a food, but it can also impart novel nutritional qualities through the introduction of live microorganisms and/or bioactive compounds generated via microbial action (2). Several studies have associated the consumption of fermented foods with positive impacts on cardiometabolic health outcomes, including improvements in body weight, modulations in blood cholesterol, and prevention of type II diabetes (3-7). However, assessment of the true intake of fermented foods is limited due to the subjective nature of many traditional dietary assessment tools (that are also not specific for assessing fermented food intake), and the lack of validation of these methods for assessing the intake of different fermented food groups.

Accurate dietary assessment is a core tenet of nutritional epidemiology that aids in the appropriate identification of diet-health associations. To date, the food frequency questionnaire (FFQ) is one of the most common dietary assessment instruments used to estimate habitual food and nutrient intake in large populations, for reference periods of one month to one year (8). Since the FFQ food list is determined based on the major foods that contribute to the total intake, as identified in food consumption surveys, it is not necessarily designed to assess the total diet (8-10). Conversely, 24-h recalls aim to assess the whole diet, but only for the previous 24 hours prior to assessment (11). In theory, multiple, non-consecutive 24-h recalls can approximate habitual intake of a food or nutrient, akin to the FFQ, but this process can be labour-intensive. Both methods rely on self-reporting, and are prone to correlated measurement and reporting errors. Nevertheless, determining the level of agreement between intakes assessed by the FFQ versus multiple 24-h recalls may provide a better approximation of 'true' dietary intake. This could help with the interpretation of the results in future studies, and avoid misidentified associations between dietary components and health.

The validity of FFQs in estimating intakes of various nutrients, foods, and food groups has been documented in multiple studies (12-16). However, to our knowledge, no groups have endeavoured to assess the validity of FFQs for estimating the intake of fermented foods. In this study, we aimed to first identify fermented foods in the diet, and subsequently assess the relative validity of a FFQ compared to multiple 24-h recalls in estimating the intake of fermented foods in a subsample of participants from a Dutch observational cohort study (NQplus). Given that the goal of nutritional epidemiological studies is to identify associations between food intake and the development of chronic diseases, the accurate assessment of dietary intake and classification of individuals into their relative levels of dietary intake is critical in order to promote accurate estimation of risk and prevent false associations.

Methods

Participants

The Nutrition Questionnaires plus (NQplus) study is a prospective cohort study that was primarily conducted in Caucasian Dutch adults (20 to 70 years), living in or around Wageningen, The Netherlands. It was initiated as an 'add-on' study to the National Dietary Assessment Reference Database (NDARD) project, to gather extensive data on participant demographics, lifestyle, medical history, and cardiometabolic health outcomes. A complete description of NQplus and the NDARD project can be found elsewhere (17, 18). Briefly, 2048 men and women were recruited and included in the study between June 2011 and February 2013. Baseline measurements included an assessment of habitual dietary intake by FFQ (n = 1468) and/or 24-h recall (n = 1117). Additional data on anthropometrics, body composition, blood pressure, pulse wave velocity, advanced

glycation endproduct (AGE) accumulation, and cognitive performance, were also collected. Background demographics, health, and lifestyle data were collected via validated questionnaires administered online using the open-source survey tool Limesurvey (Lime-Survey Project Team/Carsten Schmitz, Hamburg, Germany). Fasting blood samples and 24-h urine samples were also collected. All measurements were repeated at 1 and 2 years of follow-up and performed according to a standardised protocol by trained research personnel. The study was approved by the ethical committee of Wageningen University and Research and performed in agreement with the Declaration of Helsinki. Written informed consent were obtained from all participants prior to the start of the study.

Population for the validation study

The validation analyses were conducted with a subset of participants who had completed both a FFQ as well as 2 or more 24-h phone-based recalls. From the original dataset (n = 2048), participants who did not have any dietary assessment data were excluded (n = 17), as were those who completed fewer than two phone-based recalls (n = 1081). A further ten participants with implausible energy intakes were excluded from analyses (*i.e.*, men with energy intakes <800 or >4200 kcal/day, and women with energy intakes <500 and >3500 kcal/day) (19-22). Merging the FFQ and 24-h recall data subsets resulted in a sample of n = 809 with complete data; these participants represented the validation subcohort for further analyses.

Food frequency questionnaire (FFQ)

A full description of the dietary assessment methods have been detailed previously in the study design papers for the NQplus study and NDARD project (17, 18). The goal of NDARD was to advance the development and validation of new FFQs, while NQplus promotes research activities between dietary determinants and cardiometabolic health in Dutch adults. Habitual dietary intake was assessed using a 216-item FFQ. The food items for the FFQ were selected to cover ≥96% of the absolute level of food intake and ≥95% of the between-person variability of each nutrient under study as assessed in the 1998 Dutch National Food Consumption Survey (DNFCS), and supplemented with commonly consumed commercial food products from the 2011 DNFCS (17). The FFQ was self-administered and completed online using the open-source survey tool Limesurvey, with 10 frequency categories: never, 1 day per 4 weeks, 2-3 days per 4 weeks, 1 day per week, 2 days per week, 3 days per week, 4 days per week, 5 days per week, 6 days per week, and 7 days per week. Portion sizes were estimated using typical portion sizes and commonly used household measures. Subsequently, total food intakes (in g/day) were calculated by multiplying consumption frequency (times/day) by portion size (in grams) as defined in the Dutch food composition tables (2011) (23). It should be noted that although the reference period of the FFQ validity is one month, it was assumed that food consumption patterns are stable in this adult population. Previous validation studies for this FFQ have revealed good correlation coefficients for energy (Pearson's r=0.65 compared to 24-h recall) (24), total fats (Pearson's r=0.78 compared to dietary history) (25), as well as several micronutrients (*e.g.*, vitamin B1 and B2, Pearson's r=0.58) and food groups (*e.g.*, bread, Pearson's r=0.69) compared to the 24-h recall (15). In addition, a recent validation study evaluating a Glycaemic Index FFQ (GI-FFQ) against the general-FFQ and 24-h recalls for the NQplus cohort revealed moderate to good relative validity for carbohydrates, carbohydrate-rich foods, and glycaemic index/glycaemic load (26).

24-h Recalls

For the current analyses, we used 24-h recall data collected by telephone. The telephone-based 24-h recalls were carried out by trained dietitians and performed according to a standardised protocol (17). Portion sizes were assessed using household measures, weight/volume, and standard reference portions. Recall data were subsequently transcribed as food codes of the 2011 Dutch food composition table (23). Regular meetings with all dietitians and quality checks ensured the quality of the telephone recalls and encoding of the data.

Further information on dietary supplement intake and whether a dietary regime was followed during the month preceding the recall assessment (prescribed or at own initiative) were also recorded. The phone-based 24-h recalls were taken at the beginning of the study period, and at 6, 12, 24, and 36 months follow-up, with some participants completing less or more recalls than the indicated follow-up periods. Participants included in the validation study (n = 809) completed between two and eight phone-based 24-h recalls assessing the intake of 2102 food items. The number of participants who completed 2, 3, 4, 5, 6, 7 and 8 recalls were respectively, n = 48, 358, 53, 229, 96, 21, and 4.

Identification and classification of fermented foods

Fermented foods from the FFQ and 24-h recall food lists were identified and classified. As a first step, foods that were not consumed by any participants were removed from the analyses. This left 216 foods in the FFQ food list, and 1593 foods in the 24-h recall food list. To take into consideration the breadth of fermented foods that exist in the marketplace and in the diet, we first stratified fermented foods in the FFQ and 24-h recall food lists into broad food groups, namely dairy, meat and fish, fruits and vegetables, soya, cereals and grains, beverages, and 'other fermented products'. These food groups were defined *a priori* and were loosely based on the food-based dietary guidelines in The Netherlands, Switzerland, and United States (27-29). Fermented foods within each food group were then aggregated into subgroups. To ensure that the foods were truly fermented, a series of exclusion criteria were applied. For foods that were traditionally fermented but are typically no longer fermented due to modern food processing (*e.g.*, pickled vegetables), ingredient lists of common grocery store items were consulted, and these foods were included/excluded accordingly. Foods that contained a fermented ingredient (*e.g.*, composite dishes, such as pizza with cheese, chocolate-based confectionaries), processed variations of fermented foods (*e.g.*, chocolate spreads, cheese spreads), and foods that were not fully fermented (*e.g.*, green or black teas that are usually oxidised rather than post-fermented) were classified separately, as 'composite dishes that contain a fermented ingredient' or 'possibly fermented'.

For the validation aspects of this study, we selected fermented foods and food groups that were assessed by both the FFQ and 24-h recall methods, to enable a direct comparison between the two methods. These fermented food groups (and subgroups) included: fermented beverages (coffee, beer, and wine), fermented cereals/grains (brown bread, white bread, wholegrain bread, rye bread, or 'other bread'), fermented dairy (cheese, yoghurt, buttermilk, quark), and chocolate. Additionally, we assessed the intakes of non-fermented dairy (milk, ice cream, butter, cream) and non-fermented soya products. Intakes of these products may be closely related to the intakes of the fermented foods and thus were considered as potentially relevant for future analyses wherein associations between fermented food intake and health will be explored.

Statistical analysis

For the recalls, intakes from the total number of recalls per participant (ranging from 2 to 8) were averaged prior to statistical analysis. We calculated both absolute as well as energy-adjusted intakes for food groups, where energy-adjustment was performed using the commonly used residual method (30). In order to provide comprehensive insight into the different aspects of validity, and to reveal the limitations of each dietary assessment method, a combination of statistical tests were used to assess relative validity (31): mean percent difference, quintile cross-classification, correlation coefficient (and attenuation factors), and Bland-Altman. Group-level agreement was first assessed using mean percent difference in energy-adjusted food intake, which was calculated according to the formula:

$$Difference\ (\%) = \frac{FFQ - Recall}{Recall} * 100$$

To assess the level of agreement between intakes assessed by the two methods, quintile cross-classification was applied to the mean energy-adjusted intakes for each fermented food group. After defining the quintiles

for each food group, the percentage of individuals classified into the same, adjacent, or extreme quintile for each fermented food group was examined. If more than 50% of the participants were correctly classified in the same or adjacent quintile, with less than 5% grossly misclassified in the extreme quintile, this was interpreted as a good outcome (31, 32).

To determine the strength and direction of the associations, non-parametric Spearman's rank correlation coefficients (r_s) were calculated; correlations are shown as crude and energy-adjusted. Correlations coefficients of ≥0.50 were classified as good, 0.20 to 0.49 was considered acceptable, and <0.20 considered as poor (31). While these cut-offs are commonly used, for the 'acceptable' classification, we distinguished between a higher range (0.40 to 0.49) and lower range (0.20 to 0.39), where the higher acceptable range was considered a more rigorous cut-off to take into account the high possibility of correlated errors between the FFQ and recall methods. Attenuation factors were also calculated alongside correlation coefficients, since they are commonly used in epidemiological studies to adjust the association between diet and disease, and help indicate the extent to which diet-disease associations are weakened due to measurement error. Due to the high probability of correlated errors between the FFQ and 24-h recall methods, the attenuation factors are expected to give an incomplete correction of measurement error, and can be inflated (33, 34). Nevertheless, the use of attenuation factors (based on a 24-h recall method) has been shown to improve the relative risks of diet-disease associations (35), which warranted their inclusion in our analyses. A non-linear mixed model was used to obtain attenuation factors for all food groups. From the model parameters, we calculated the attenuation factors (λ_x) using the 24-h recall as a reference method according to methods previously described by Trijsburg *et al.* (36), and specified in the formula:

$$\lambda_X = \frac{\beta_x * varT}{{\beta_x}^2 * varT + \frac{var\ \varepsilon_{Xij}}{k} + varw_{xi}}$$

where β_X is the proportional scaling bias of the reference method (X), varT is the variance of the true intake, $var\varepsilon_{Xij}$ is the variance of the random error of the reference method, and $varw_{xi}$ indicates the variance of the person-specific bias of the reference method. To obtain the estimates of the attenuation factor for multiple 24-h recalls, the variance of the random error of the method ($var\varepsilon_{Xij}$) was divided by the number of measurements (k) of the reference method.

Finally, Bland-Altman plots were constructed to examine the group-level agreement between the FFQ and recall (*i.e.*, mean of multiple recalls) by plotting the mean measure [(FFQ+Recall)/2] against the difference in measures (FFQ-Recall) (37). To visually assess the degree of error, additional analyses were added to the plots, including: a line indicating the mean difference, and upper and lower 95% confidence intervals [mean ± (standard deviation of the mean difference*1.96)]. Additional regression analyses were conducted to detect proportional biases, and evaluate the direction and magnitude of the bias. All analyses were conducted in R, version 3.5.0 (38), with the exception of quintile cross-classification and Bland-Altman analyses, which were conducted using the statistical package IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA), and the attenuation factors, which were calculated using SAS, version 9.3 (SAS Institute Inc. Cary, NC, USA, 2012). The level of statistical significance was set as $p \leq 0.05$.

Results

Participants in the validation study

The characteristics of the participants included in the validation study are shown in **Table 1**. Participants had a mean age of 53 ± 12 years, and 53% of the population were men. Approximately 48% of participants (39% of men and 58% of women) had a body mass index (BMI) below 25 kg/m^2, while 52% were overweight or obese (61% of men and 42% of women). The majority of participants had a high educational

level (65%), had never smoked (52%), and had not followed a diet in the month preceding enrolment to the study (93%). About a fifth of participants had a disease history of hypertension (22%) and high cholesterol (18%), while only a small percentage (less than 5%) had a history of cancer, diabetes, heart attack, and/or stroke.

Table 1. General characteristics of the participants included in the validation study

	All (*n* = 809)	Men (*n* = 425)	Women (*n* = 384)
Age, years	53.1 ± 11.9	55.5 ± 11.0	50.4 ± 12.2
BMI, kg/m^2	25.6 ± 3.8	26.1 ± 3.3	25.0 ± 4.2
BMI category			
<25 kg/m^2	387 (48)	165 (39)	222 (58)
≥25 kg/m^2	421 (52)	259 (61)	162 (42)
Waist circumference, cm	90.7 ± 12.0	95.9 ± 10.3	84.8 ± 11.1
Education, n (%)			
Low	54 (7)	31 (7)	23 (6)
Intermediate	223 (28)	109 (26)	114 (30)
High	529 (65)	285 (67)	244 (64)
Smoking status, n (%)			
Never	364 (52)	170 (45)	194 (60)
Former	270 (39)	163 (44)	107 (33)
Current	65 (9)	41 (11)	24 (7)
Disease history, n (%)			
Cancer	44 (5)	17 (4)	27 (7)
Diabetes	21 (3)	15 (4)	6 (2)
Heart attack	16 (2)	12 (3)	4 (1)
Hypertension	179 (22)	101 (24)	78 (20)
High cholesterol	147 (18)	92 (22)	55 (14)
Stroke	9 (1)	8 (2)	1 (0)
Diet during month preceding study, n (%)			
No	749 (93)	402 (94)	347 (91)
Yes, always	28 (3)	8 (2)	20 (5)
Yes, sometimes	31 (4)	15 (4)	16 (4)
Energy, kcal/day			
FFQ	2143.7 ± 504.8	2344.6 ± 509.7	1921.3 ± 394.5
24-h Recall[a]	2129.2 ± 444.2	2315.2 ± 451.2	1923.4 ± 331.5
Protein, g/day			
FFQ	77.4 ± 17.7	83.4 ± 17.7	70.7 ± 15.1
24-h Recall[a]	82.6 ± 18.4	89.8 ± 18.6	74.6 ± 14.5
Fat, g/day			
FFQ	85.4 ± 25.9	93.1 ± 27.1	76.9 ± 21.6
24-h Recall[a]	81.6 ± 21.7	87.7 ± 22.8	74.7 ± 18.1
Carbohydrates, g/day			
FFQ	231.6 ± 61.1	251.7 ± 63.5	209.4 ± 49.6
24-h Recall[a]	230.8 ± 58.0	249.1 ± 62.2	210.5 ± 44.8
Fibre, g/day			
FFQ	25.0 ± 6.8	26.1 ± 7.3	23.8 ± 5.9
24-h Recall[a]	23.4 ± 6.8	24.5 ± 7.1	22.1 ± 6.2

BMI, body mass index; FFQ, food frequency questionnaire; SD, standard deviation.
Values are presented as mean ± SD, unless otherwise specified. Missing values: BMI (*n* = 1), waist circumference (*n* = 1), education (*n* = 3), smoking status (*n* = 110), diet during last month (*n* = 1).
[a] Mean of multiple 24-h recalls.

Identification of fermented foods in the diet and comparison of mean intakes

The identification and classification of fermented foods from the FFQ and 24-h recall into food groups and subgroups, is provided in **Table S1**. For the FFQ, 39 foods (18%) were classified as fermented, including 5 types of fermented beverages, 12 types of fermented cereals/grains, 3 types of chocolate, 17 types of fermented dairy products, and 2 other fermented products. A further 19 (9%) of foods in the FFQ food list were classified as 'composite dishes that contain a fermented ingredient' or 'possibly fermented'. For the 24-h recall, 247 foods (16%) were classified as fermented, including 20 types of fermented beverages, 95 types of fermented cereals/grains, 20 types of cocoa products, 96 types of fermented dairy, 4 types of fermented fruits/vegetables, 6 types of fermented meat/fish, 4 types of fermented soya, and 2 other fermented products. A further 228 (14%) of foods in the recall food list were classified as 'composite dishes containing a fermented ingredient' or 'possibly fermented'.

Mean energy-adjusted daily intakes and percentage of average daily energy intake for each fermented food group, the number of consumers per food group, as well as the percent and absolute differences in mean intakes, are presented in **Table 2**. The mean daily energy intake as estimated by the FFQ was 2144 (±505) kcal/day, which was comparable to the energy intake estimated by the 24-h recalls of 2129 (±444) kcal/day (0.68% difference). Fermented food groups with the highest intakes for both the FFQ and 24-h recall were total fermented beverages (respectively, 606 and 610 g/day; the main contributor was coffee), fermented dairy (respectively, 171 and 176 g/day; the main contributor was yoghurt), and fermented cereals/grains (respectively, 129 and 143 g/day; the main contributor was wholegrain bread). When expressed as a percentage of average daily energy intake, the main contributor changed for total fermented beverages to wine (respectively, 2.6 and 2.8%, for the FFQ and 24-h recall), and for total fermented dairy to cheese (respectively, 4.9 and 5.2%). For fermented cereals/grains, the main contributor remained wholegrain bread (respectively, 9.5 and 9.3%). Taking into account all fermented food groups, the mean percent (and absolute) difference between the FFQ and the 24-h recall data ranged from 0.3% (0.1 g/day) for cheeses to 10224.4% (41.9 g/day) for buttermilk. Mean intakes were similar between the FFQ and 24-h recall methods for total fermented beverages (percent difference of -0.7%) and in particular coffee (2.1%), fermented cereals and grains (-9.7%), with smaller differences for specific assessments of brown bread, wholegrain bread, and rye breads, and total fermented dairy (-2.8%), particularly for cheeses (-0.3%). On the contrary, percent differences in mean intake for buttermilk, quark, and white bread were large (≥53%).

High intake levels of non-fermented dairy foods were also observed in this population (recall 137g/d and FFQ 153 g/day), the main contributor being milk (**Table 2**). While the percent difference in mean intakes was similar for butter (0.7%), a larger difference was observed for cream (-62.5%) and non-fermented soya (-15.9%).

Compared to group level percent differences in means, higher individual level percent differences in means was observed for total energy as well as multiple fermented food groups, with the most striking contrasts observed for beer, brown bread, white bread, rye bread, 'other bread', fermented dairy, cheeses, yoghurts, and buttermilk (**Table S2**). Meanwhile, for wine, total fermented cereals/grains, quark, and ice cream, the mean percent differences on an individual level were improved. From the non-fermented food groups evaluated, milk and soya had large differences between percent differences in means determined on an individual compared to group level.

Table 2. Mean intake of fermented and non-fermented products assessed by FFQ and 24-hour recalls

	FFQ			24-hour Recall			% Difference in group means (absolute difference)[b]
	Mean[a]	SD	Consumers	Mean[a]	SD	Consumers	
Energy, kcal/day	2143.7	504.8	809	2129.2	444.2	809	0.7 (14.5)
Fermented beverages	605.5 (4.5%)	376.7	782	609.6 (5.5%)	371.8	774	-0.7 (-4.1)
Coffee	457.6 (0.2%)	303.0	744	448.3 (0.8%)	287.9	742	2.1 (9.3)
Beer	82.8 (1.7%)	163.0	436	85.7 (1.8%)	174.4	267	-3.3 (-2.9)
Wine	65.0 (2.6%)	88.5	613	75.3 (2.8%)	100.0	507	-13.7 (-10.3)
Fermented cereals/grains	128.7 (15.0%)	52.1	806	142.6 (17.3%)	52.7	805	-9.7 (-13.9)
Brown bread	23.9 (2.6%)	36.0	535	24.6 (2.9%)	33.1	464	-2.6 (-0.7)
White bread	8.7 (1.1%)	15.6	531	18.6 (2.3%)	29.2	418	-53.4 (-9.9)
Wholegrain bread	82.4 (9.5%)	52.4	760	79.4 (9.3%)	55.5	723	3.8 (3)
Rye bread	3.3 (0.3%)	9.5	262	3.2 (0.3%)	11.7	103	5.1 (0.1)
Other bread	8.6 (1.1%)	14.3	274	7.7 (1.0%)	15.5	271	10.8 (0.9)
Pastries	1.8 (0.4%)	4.3	368	2.2 (0.4%)	6.7	117	-16.4 (-0.4)
Chocolate	5.6 (1.4%)	7.9	715	9.0 (2.2%)	10.8	575	-37.4 (-3.4)
Fermented dairy	170.6 (8.2%)	125.3	804	175.5 (8.5%)	129.8	795	-2.8 (-4.9)
Cheeses	31.6 (4.9%)	25.2	785	31.7 (5.2%)	20.4	761	-0.3 (-0.1)
Yoghurts	93.7 (2.5%)	90.5	704	81.8 (2.0%)	90.3	586	14.6 (11.9)
Quark	3.0 (0.1%)	16.5	60	13.9 (0.5%)	34.2	207	-78.6 (-10.9)
Buttermilk	42.3 (0.6%)	75.1	316	0.4 (0.01%)	7.3	3	10224.4 (41.9)
Non-fermented dairy	152.7 (5.5%)	136.1	802	136.8 (5.5%)	132.4	752	11.7 (15.9)
Butter	3.0 (1.0%)	7.7	309	3.0 (1.0%)	6.0	308	0.8 (0)
Cream	3.0 (0.4%)	7.1	685	7.9 (0.7%)	13.4	444	-62.5 (-4.9)
Ice cream	6.1 (0.7%)	9.1	520	7.8 (0.9%)	14.7	242	-22.0 (-1.7)
Milk	140.7 (3.5%)	136.1	691	115.6 (2.6%)	131.9	591	21.7 (25.1)
Non-fermented soya	9.3 (0.3%)	34.3	683	11.1 (0.3%)	46.4	92	-15.9 (-1.8)

FFQ, food frequency questionnaire; SD, standard deviation.

[a] Mean energy-adjusted intakes for the entire validation sample. Values are in g/day (and as % average daily energy intake) (n = 809).

[b] Percent difference is calculated using [(FFQ - Recall)/Recall] x 100% for each food or food group. For comparison, the absolute difference (FFQ – Recall) is also provided.

Quintile cross-classification

The degree of potential misclassification of fermented foods was examined using quintile cross-classification (**Table 3**). All fermented food groups were characterised by over 50% of participants being classified into the same or adjacent quintile of intake, confirming good ranking ability (ranging from 57.8% for buttermilk to 88.5% for total fermented beverages). Furthermore, for total fermented beverages, coffee, and wine, almost 50% of participants were classified in the same quintile for both methods. While misclassification in the extreme quintiles was relatively low across the total fermented food groups (0.4-3.8%), a greater proportion of participants (5.3 to 6.8%) were grossly misclassified for some individual fermented foods including brown bread, 'other bread', pastries, quark, and buttermilk.

Non-fermented dairy and soya food groups also had good agreement between dietary assessment tools in the quintile cross-classification, with over 50% of participants classified into the same or adjacent quintile of intake (**Table 3**). However, for cream and ice cream, a relatively higher percentage (5.4 and 5.7%, respectively) were misclassified into the extreme quintiles.

Table 3. Quintile cross-classification and Spearman's correlations for fermented and non-fermented foods

Food Group	Agreement of Quintiles for Food Group Intake[a]			Spearman's Rank Correlation Coefficient (r_s)	
	Same Quintile (%)	Adjacent Quintile (%)	Extreme Quintile (%)[b]	Crude	Energy-Adjusted
Fermented beverages	47.6	40.9	0.4	0.80**	0.78**
Coffee	48.8	37.2	0.9	0.76**	0.74**
Beer	39.9	35.0	1.7	0.67**	0.53**
Wine	46.4	38.9	0.3	0.76**	0.74**
Fermented cereals/grains	38.1	41.3	1.2	0.68**	0.63**
Brown bread	30.3	34.9	6.2	0.25**	0.28**
White bread	35.2	34.5	4.7	0.33**	0.35**
Wholegrain bread	38.8	37.9	1.9	0.61**	0.55**
Rye bread	33.9	34.2	3.0	0.43**	0.42**
Other bread	28.1	32.1	6.8	0.11**	0.17**
Pastries	29.2	33.7	5.4	0.20**	0.27**
Chocolate	27.8	39.8	3.8	0.36**	0.38**
Fermented dairy	43.1	40.2	0.9	0.68**	0.69**
Cheeses	32.9	38.1	2.2	0.46**	0.47**
Yoghurts	34.5	41.5	1.7	0.56**	0.55**
Quark	30.2	36.3	5.3	0.13**	0.31**
Buttermilk	25.2	32.6	5.6	0.10**	0.18**
Non-fermented dairy	40.9	41.5	0.9	0.68**	0.67**
Butter	36.6	37.6	2.5	0.48**	0.51**
Cream	28.7	30.5	5.4	0.20**	0.21**
Ice cream	26.1	35.1	5.7	0.23**	0.21**
Milk	40.9	41.9	1.1	0.67**	0.66**
Non-fermented soya	34.1	36.0	4.1	0.40**	0.41**

FFQ, food frequency questionnaire. **, $p<0.01$.

[a] Mean energy-adjusted intake values for each food group were used to divide participants into quintiles.

[b] Percentage of 1st quintile participants in the FFQ classified into the 5th quintile in the recall, or vice versa.

Spearman's correlations

Crude Spearman's correlation coefficients ranged from to 0.10 (buttermilk) to 0.80 (fermented beverages) (**Table 3**). Energy-adjustment slightly increased the correlation coefficient for brown bread, white bread, 'other bread', pastries, chocolate, total fermented dairy, cheeses, quark, and buttermilk, and slightly decreased the correlation coefficient for other fermented food groups. Strong correlations ($r_s \geq 0.50$) for both crude and energy-adjusted intakes were obtained for fermented beverages (including coffee, beer, wine), fermented cereals/grains (including wholegrain bread), and fermented dairy (including yoghurts). Correlation coefficients in the higher acceptable range were obtained for rye bread and cheeses ($0.40 \leq r_s \leq 0.49$), while correlation coefficients in the lower acceptable range were found for brown bread, white bread, pastries, and chocolate ($0.20 \leq r_s \leq 0.39$). Only for three fermented foods ('other bread', quark, buttermilk) was the correlation coefficient was less than 0.20. All correlations were statistically significant ($p<0.01$). Crude and energy-adjusted attenuation factors for two 24-h recall replicates were consistently high for fermented beverages (0.81), coffee (0.85), beer (0.71), wine (0.64), while moderate values were observed in the range of attenuation factors for fermented cereals/grains (0.51), wholegrain bread (0.47), rye bread (0.54), fermented dairy (0.52), cheese (0.42), and yoghurts (0.53) (**Table S3**). In comparison, lower values in the range of attenuation factors were obtained for brown bread (0.28), white bread (0.26), 'other bread' (0.19), pastries (0.23), chocolate (0.32), and quark (0.14). For buttermilk, accurate attenuation factors could not be calculated due to the low variance of the person-specific biases compared to the within- and between-person variances. As expected, for all food groups, attenuation factors improved with increasing replicates of the reference 24-h recall from two (0.14-0.86) to eight (0.24-1.0). Energy-adjustment had little effect on the attenuation factors. Sex-specific correlation coefficients (crude and energy-adjusted) were similar compared to those obtained for the total population, as well as between men and women, for virtually all fermented food groups (**Table S4**). Energy-adjustment generally had a negligible effect on the sex-specific correlation coefficients, but were amplified for less commonly consumed foods, in the positive (*i.e.*, other bread and buttermilk in men, quark and other bread in women) or negative direction (*i.e.*, rye bread and quark in men, buttermilk in women).

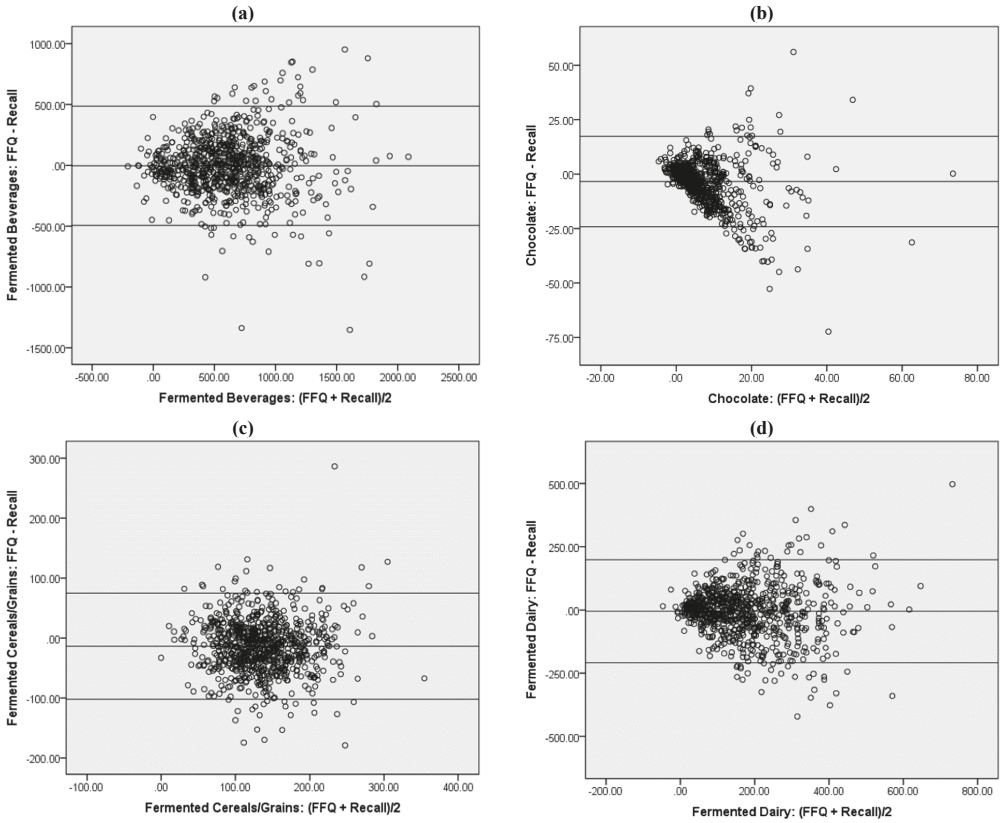
Similarly, for non-fermented food groups, strong correlations were obtained for non-fermented dairy, including milk, while acceptable correlations were obtained for butter and non-fermented soya (in the higher range), and ice cream and cream (in the lower range) (**Table 3**). The crude and energy-adjusted attenuation factors obtained were high for non-fermented dairy (0.69-0.98) and butter (0.65-0.97), moderate for milk (0.57-0.72) and non-fermented soya (0.6-0.76), and lower for cream (0.32-0.5) and ice cream (0.18-0.63) (**Table S3**). Sex-specific correlation coefficients (crude and energy-adjusted) for non-fermented food groups were similar compared to those obtained for the total population, as well as between men and women (**Table S4**).

Bland-Altman analyses

The results of the Bland-Altman analyses revealed good agreement in group-level intakes for total fermented beverages, including coffee and beer (**Table S2**). Good agreement was also demonstrated for brown bread, wholegrain bread, rye bread, 'other bread', pastries, chocolate, fermented dairy, and cheeses (mean difference between -0.1 to 9.3 g/day; $p_{difference} \geq 0.05$). However, for wine, total fermented cereals/grains, white bread, chocolate, yoghurts, quark, and buttermilk, significant differences were found between the two dietary assessment methods (mean difference between -3.4 to 41.9, $p_{difference}<0.0001$). The results of the regression analyses further revealed a significant amount of proportional bias for wine, white bread, rye bread, pastries, chocolate, cheese, quark, and buttermilk ($p_{slope}<0.0001$). For certain foods (wine, white bread, pastries, chocolate, cheese, and quark), the FFQ tended to consistently underestimate their consumption compared to the 24-h recalls, while intakes were overestimated for others (rye bread and buttermilk). A small bias for coffee, beer, brown bread, and 'other bread' was also observed ($p<0.05$) (**Table S2**). These results were also confirmed visually in the Bland-Altman plots for the main fermented food groups (**Figure 1**) and subgroups (**Figure S1**).

For non-fermented foods, good agreement between dietary assessment methods was demonstrated for butter and non-fermented soya (mean difference between 0.02 to -1.8; $p \geq 0.05$), while poor agreement was revealed for total non-fermented dairy, cream, ice cream, and milk (mean difference between -4.9 to 25.1; $p_{difference} < 0.0001$) (**Table S2**). The results of the regression analyses demonstrated significant proportional bias for butter, cream, ice cream, and non-fermented soya ($p_{slope} < 0.0001$) (**Table 1** and **Figure S2**).

Figure 1. Bland-Altman plots demonstrating relative validity of FFQ versus 24-h recalls for main fermented food groups. Group-level relative validity assessed for: (a) fermented beverages, (b) fermented cereals/grains, (c) chocolate, and (d) fermented dairy. The middle line indicates the mean difference, while the upper and lower lines indicate the 95% confidence intervals, respectively [calculated as: mean ± (standard deviation of the mean difference X 1.96)].



Summary of Validity Assessment

A summary assessment of the different aspects of validity between the FFQ and 24-h recall methods is provided in **Table 4**.

Table 4. Summary of validity assessments between the FFQ and 24-hour recalls for fermented and non-fermented foods

Method	% Difference	Correlation Coefficient	Quintile Cross-Classification	Bland-Altman[a]
Aspect of Validity Measured	Agreement (group level)	Strength and direction of association (individual level)	Agreement (individual level)	Presence, direction, and extent of bias (group level)
Interpretation Criteria	Acceptable: 0.0 to 10.0% Poor: >10%	Good: ≥0.50 Acceptable: 0.20 to 0.39 (lower range); 0.40 to 0.49 (higher range) Poor: <0.20	Good: ≥50% in same/adjacent quintile; ≤5% in extreme quintile Poor: <50% in same/adjacent quintile; >5% in extreme quintile	Good: p>0.05 Poor: p≤0.05
Fermented beverages	**Acceptable**	**Good**	**Good**	**Good**
Coffee	**Acceptable**	**Good**	**Good**	**Good (bias)**
Beer	Poor	**Good**	**Good**	**Good (bias)**
Wine	Poor	**Good**	**Good**	Poor (bias)
Fermented cereals/grains	**Acceptable**	**Good**	**Good**	Poor
Brown bread	**Acceptable**	**Acceptable (low)**	Poor	**Good (bias)**
White bread	Poor	**Acceptable (low)**	**Good**	Poor (bias)
Wholegrain bread	**Acceptable**	**Good**	**Good**	**Good**
Rye bread	**Acceptable**	**Acceptable (high)**	**Good**	**Good (bias)**
Other bread	Poor	Poor	Poor	**Good (bias)**
Pastries	Poor	**Acceptable (low)**	Poor	**Good (bias)**
Chocolate	Poor	**Acceptable (low)**	**Good**	Poor (bias)
Fermented dairy	**Acceptable**	**Good**	**Good**	**Good**
Cheese	**Acceptable**	**Acceptable (high)**	**Good**	**Good (bias)**
Yoghurts	Poor	**Good**	**Good**	Poor
Quark	Poor	Poor	Poor	Poor (bias)
Buttermilk	Poor	Poor	Poor	Poor (bias)
Non-fermented dairy	Poor	**Good**	**Good**	Poor
Butter	**Acceptable**	**Acceptable (high)**	**Good**	**Good (bias)**
Cream	Poor	**Acceptable (low)**	Poor	Poor (bias)
Ice cream	Poor	**Acceptable (low)**	Poor	Poor (bias)
Milk	Poor	**Good**	**Good**	Poor
Non-fermented soya	Poor	**Acceptable (high)**	**Good**	**Good (bias)**

FFQ, food frequency questionnaire. Acceptable and good validity assessment outcomes are bolded.

[a] The presence of proportional bias for each food group is indicated in brackets.

Discussion

Consumption of fermented foods by adults in the Netherlands

While it has been previously estimated that 5 to 40% of foods in the human diet are fermented (39), a quantitative evaluation of the contribution of fermented foods to the human diet had not been conducted prior to this report. Based on the present analysis, approximately 16 to 18% of foods consumed in this population are fermented food items, while a further 9 to 14% are composite dishes that contain a fermented ingredient, indicating that there is a high prevalence of fermented foods in the Dutch diet. These estimates are also likely to be valid for other countries (in Europe or worldwide) in which primarily Western diets are consumed.

Reliability of the current FFQ for estimating fermented food intake

In the present study, we also assessed the relative validity of a FFQ compared to multiple 24-h recalls for estimating the intake of fermented foods in a Dutch adult population. Using a combination of validation methods, including percent difference, quintile cross-classification, Spearman's correlation, and Bland-Altman plots, fermented food groups that had acceptable or good validity across all measures included total fermented beverages, coffee, wholegrain bread, rye bread, fermented dairy, and cheese. From the non-fermented food groups that were assessed, butter was the only food with uniformly good/acceptable validity. In addition, wine, beer, fermented cereals/grains, white bread, chocolate, yoghurts, non-fermented dairy, milk, and non-fermented soya all had good ranking ability (as indicated by the strong correlation coefficients and high agreement in quintile cross-classification), albeit poor parametric assessment of differences (as indicated by the low agreement in percent difference and Bland-Altman).

Fermented foods with the highest consumption levels included coffee (~453 g/day; ~0.5% of daily energy intake), yoghurts (~88 g/day; ~2.3% of daily energy intake), beer (~84 g/day; ~1.8% of daily energy intake), wholegrain bread (~81 g/day; ~9.4% of daily energy intake), wine (~65 g/day; ~2.7% of daily energy intake), and cheese (~32 g/day; ~5.1% of daily energy intake). These foods, with the exception of coffee, also correspond to the top fermented foods contributing to total daily energy intake in this study. Comparing our findings to studies in other European populations, mean daily intakes were similar for coffee (404 g/day), bread products (64 to 146 g/day), butter (5 g/day), cheese (25 to 58 g/day), yoghurt (95 g/day), and soya products (6 to 10 g/day) (12, 13, 15, 40); however, milk consumption in our study is a little lower than previously reported (220 to 230 g/day) (12, 13, 15). As indicated previously, the comparison of mean energy-adjusted daily intakes for the fermented food groups revealed group-level differences ranging from 0.3% (for cheeses) to 10224% (for buttermilk). Foods that are consumed by the majority of the population on a regular basis, such as coffee, bread, and cheeses, showed comparable intakes for 24-recall and FFQ assessments (-0.3 to 9.7%), which was expected. The most striking differences in mean intakes were for buttermilk and quark (10224 and -78.6%, respectively), which might be a consequence of the difference in number of consumers between the FFQ and recall for these foods (60 vs. 207 for quark, 316 vs. 3 for buttermilk). Moreover, the results of both comparison of mean intakes and Bland-Altman revealed that intakes for most fermented food groups were slightly underestimated by the FFQ when compared to the 24-h recalls, which is expected since the 24-h recall, by design, generally captures a greater proportion of the diet than the FFQ.

Since a critical measure of success for an FFQ is its ability to accurately rank individuals into high- and low-intakes based on their habitual diet (13), we evaluated ranking ability using both Spearman's correlation and quintile cross-classification. High Spearman's correlation coefficients ($r_s \geq 0.50$) were obtained for all fermented beverage groups (total fermented beverages, coffee, beer, wine), total fermented cereals/grains, wholegrain bread, total fermented dairy, yoghurts, total non-fermented dairy, and milk. Since the use of different dietary assessment instruments in distinct populations could affect results, we compared

our results with those obtained from other studies for similar food groups. Streppel *et al.* (15) assessed the relative validity of a previous version of the FFQ used in the current study in 128 elderly Dutch individuals. Comparing the FFQ data to three 24-h recalls, Pearson's correlations of 0.71 to 0.93 for bread, 0.46 to 0.61 for cheese, 0.68 to 0.75 for milk and milk products, and 0.50 to 0.66 for soya and vegetarian products were obtained. Similar validation studies have been conducted in 161 German adults (12), 100 Belgian adults (14), 1,213 German adolescents (13), and 56 Swiss adults (40), comparing a FFQ to multiple 24-h recalls, 7-day estimated diet records, diet history interviews, and 4-day weighted food records, respectively. Collectively, the correlations obtained in these studies of 0.69 to 0.78 for coffee, 0.40 to 0.42 for dairy products, 0.63 for butter, 0.63 to 0.66 for milk, 0.49 for curd cheese, soured milk, and yoghurt, 0.25 to 0.61 for cheese, 0.59 for ice cream, and 0.16 to 0.48 for bread and cereals, are similar to those determined in the current study (0.78 to 0.80 for coffee, 0.20 to 0.21 for butter, 0.66 to 0.67 for milk, 0.46 to 0.47 for cheese, 0.55 to 0.56 for yoghurt, 0.21 to 0.23 for ice cream, and 0.63 to 0.68 for total fermented cereals/grains). The interesting exception is that the correlation coefficients determined for total fermented cereals/grains (r_s 0.63) and wholegrain bread (r_s 0.55) in our study are slightly lower than those reported in Streppel *et al.* (15) (r_s 0.71 to 0.93 for bread). Although total bread consumption in this elderly population (126 to 133 g/day) is comparable to total fermented cereals/grains intake by the study cohort described in the current publication (129 to 143 g/day), older adults tend to have more stable diets and different dietary patterns than those of younger adults, which might account for this difference in correlation coefficients. The attenuation factors obtained for the food groups investigated in this study were considerably higher for fermented beverages, coffee, beer, and wine (range between 0.64-1.0) than for brown bread, white bread, 'other bread', pastries, chocolate, and quark (range between 0.14-0.77). Since attenuation factors closer to one indicates a better overall estimation of intake (36), these results suggest that intake estimates for all fermented beverages are reliable across the dietary methods used here. Meanwhile, intake estimates were weaker for fermented cereals and grains, wholegrain bread, rye bread, fermented dairy, cheeses, and yoghurts, and weakest for brown bread, white bread, 'other bread', pastries, chocolate, and quark. These effects correspond with other assessments of validity for the food groups investigated.

Our results for quintile cross-classification further supported a high level of agreement between the FFQ and 24-h recall in ranking participants for the majority of fermented foods, non-fermented dairy, and soya, even when using more stringent criteria of quintiles and cut-offs of 5% for misclassification. Other validation studies have reported similar cross-classifications for cheese (46.8% same quartile, 50% same tertile), milk (45.6-49.5% same quartile), milk and soya products (57.4% same tertile), butter (49.7-50.3% same quartile) (12-14), and slightly lower cross-classification for coffee (75.2% same quartile) and ice cream (44.1% same quartile) (13). Interestingly, in these studies and ours, bread products had a lower accurate classification rate (33.1-39.2% in same tertile or quartile, and 6.4-8.4% in opposite quartile (12-14); 28.1-38.8% in same quintile and 1.2-6.8% in opposite quintile (present study)). Although total fermented cereals/grains had good cross-classification, the same is not true for the corresponding subgroups, which may be also be attributed to the misclassification of bread products by consumers for FFQs. Sporadically consumed foods (*e.g.*, quark) or seasonal foods (*e.g.*, ice cream) also tended to have a higher discordance and degree of misclassification between methods. However, these results were expected due to the lower probability of assessing such foods on recall days, compared to the FFQ which evaluates a larger reference period (12).

Study strengths and limitations

A strength of this study is the inclusion of a large population size for validation. Moreover, we utilized multiple assessment methods, which allows for a more comprehensive evaluation of relative validity at both the individual and group level (31). Notwithstanding, there are several limitations to address, the most dominant of which relate to the inherent limitations associated with using the FFQ and 24-h recall to measure dietary intake. Firstly, in an ideal validation, the comparison and reference methods should have independent

error sources (9). Both the FFQ and 24-h recall rely on the memories and perceptions of the participants, which can lead to higher estimates of validity (13). Moreover, both methods rely on the same food composition table and tools to classify foods and quantify portion sizes, as an additional source of correlated error. Thus, only relative validity could be determined in this study. Secondly, estimates of portion sizes were performed using standard portion sizes and household measures. While these are commonly used approaches, different interpretations of household portion sizes can lead to misclassification and measurement bias. In particular, this misclassification may be more pronounced in estimates of absolute intake (*i.e.*, in g/day), while a small effect is anticipated on the ranking ability of the FFQ (*i.e.*, into quintiles of intake). Ongoing innovations in technology-based tools are aimed to reduce this source of measurement bias in dietary assessment. Thirdly, foods that are not frequently consumed or consumed only seasonally may be inconsistently or unevenly captured, depending on when the diet was assessed. Fourthly, since the dietary assessments in NQplus were performed from 2011 to 2014, we cannot exclude the possibility of changes in diet that would obscure the underlying assumption that participants have very stable diets at the time of assessment.

A further aspect worth highlighting is that the FFQ used in the NQplus study was not specifically designed to assess the intake of fermented foods; consequently, not all fermented food products could be captured by this method and some may only be represented by a food group comprising fermented and non-fermented foods. Indeed, 247 fermented foods were reported for the 24-h recall compared to only 39 in the FFQ. The fermented foods in this validation study primarily consisted of foods common to the Western diet (*e.g.*, coffee, breads, dairy), but there exists a wealth of fermented foods from other cultures and regions that are increasingly consumed due to globalisation (*e.g.*, kombucha, kefir, kimchi, natto, tempe). Novel fermented foods are also being developed, driven by the realisation of their potential impacts on health (41), and that consumption of fermented foods with live microorganisms may promote a healthy gut microbiota (42, 43). Conversely, some traditionally fermented products (*e.g.*, pickles, olives, mozzarella) are no longer fermented due to modernisation of food processing technologies (44). As such, fermented and non-fermented versions of the same products become indistinguishable to consumers. This emphasises the importance for developing a FFQ specific for fermented food products. Nevertheless, the present study reveals the strengths and limitations of each tool for assessing fermented food intake, highlights the need to design a FFQ to specifically assess fermented food intake, and aids in our goal of developing unbiased biomarkers of intake for fermented foods. Improved dietary assessment of fermented foods is expected to aid future trials that investigate associations between fermented food intake and (cardiometabolic) health.

Conclusions

About a fifth of the Dutch diet consists of fermented food items. Adequate to good relative validity was determined for the FFQ compared to the 24-h recall across all statistical measures for commonly consumed foods, including total fermented beverages, coffee, wholegrain bread, rye bread, total fermented dairy, cheeses, as well as butter (non-fermented). For wine, beer, total fermented cereals/grains, white bread, chocolate, yoghurts, as well as total non-fermented dairy, milk, and non-fermented soya, good ranking ability of participants into their levels of consumption could be established, albeit poor agreement in absolute intakes. For quark and buttermilk (fermented), as well as cream and ice cream (non-fermented), acceptable relative validity between the two methods could not be established; thus, the intakes for these food groups should be interpreted with caution in future studies using this population. Developing a FFQ specific for fermented foods would be valuable to capture global fermented foods that are increasingly consumed, and to delineate between fermented and non-fermented versions of foods that could obscure investigations between fermented food intake and health.

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List of abbreviations

DNFCS, Dutch National Food Consumption Survey; FFQ, food frequency questionnaire; NDARD, National Dietary Assessment Reference Database; NQplus, Nutrition Questionnaires plus.

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Supplementary Materials

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]

Food[b]	Fermentation Status	Food Group	Subgroup	(Main) Fermentation Organism	Mean intakes (g/day)
Food Frequency Questionnaire					
Beer	Fermented	Beverages	Beer	Yeast	83.69
Low-alcohol beer	Fermented	Beverages	Beer	Yeast	3.92
Coffee	Fermented	Beverages	Coffee	Endogenous	452.67
Wine, other	Fermented	Beverages	Wine	Endogenous	19.24
Wine, red	Fermented	Beverages	Wine	Endogenous	46.29
Brown bread	Fermented	Cereals/grains	Bread, brown	Yeast	3.18
Brown bread (sliced)	Fermented	Cereals/grains	Bread, brown	Yeast	21.54
Bread with raisins	Fermented	Cereals/grains	Bread, other	Yeast	8.63
Rye bread	Fermented	Cereals/grains	Bread, rye	Yeast	2.91
Biscuit (bread), white	Fermented	Cereals/grains	Bread, white	Yeast	0.69
White bread	Fermented	Cereals/grains	Bread, white	Yeast	3.35
White bread (sliced)	Fermented	Cereals/grains	Bread, white	Yeast	4.57
Biscuit (bread), whole wheat	Fermented	Cereals/grains	Bread, wholegrain	Yeast	1.69
Multiple grain bread	Fermented	Cereals/grains	Bread, wholegrain	Yeast	2.66
Multiple grain bread (sliced)	Fermented	Cereals/grains	Bread, wholegrain	Yeast	29.74
Whole wheat bread (sliced)	Fermented	Cereals/grains	Bread, wholegrain	Yeast	46.89
Croissant	Fermented	Cereals/grains	Pastry	Yeast	1.85
Milk chocolate	Fermented	Cocoa	Chocolate	Endogenous	1.94
Pure chocolate	Fermented	Cocoa	Chocolate	Endogenous	2.94
White chocolate	Fermented	Cocoa	Chocolate	Endogenous	0.53
Buttermilk	Fermented	Dairy	Buttermilk	Bacteria	43.46
Low-fat cheese (20+/30+)	Fermented	Dairy	Cheese, hard	Bacteria	5.12
Regular cheese (40+)	Fermented	Dairy	Cheese, hard	Bacteria	1.67
Regular cheese (48+)	Fermented	Dairy	Cheese, hard	Bacteria	12.47
Cheese as snack	Fermented	Dairy	Cheese, unspecified	Bacteria	3.23
Cheese with hot meal	Fermented	Dairy	Cheese, unspecified	Bacteria	3.08
Fat luxury cheese	Fermented	Dairy	Cheese, unspecified	Bacteria	1.86
Less-fat luxury cheese	Fermented	Dairy	Cheese, unspecified	Bacteria	1.99
Unknown cheese	Fermented	Dairy	Cheese, unspecified	Bacteria	0.58
(Fruit) quark with breakfast	Fermented	Dairy	Quark	Bacteria	3.43
Full (fruit) yoghurt	Fermented	Dairy	Yoghurt	Bacteria	4.45
Full yoghurt	Fermented	Dairy	Yoghurt	Bacteria	12.08
Semi-skim (fruit) yoghurt	Fermented	Dairy	Yoghurt	Bacteria	5.73
Semi-skim yoghurt	Fermented	Dairy	Yoghurt	Bacteria	24.75
Skim (fruit) yoghurt	Fermented	Dairy	Yoghurt	Bacteria	13.51
Skim yoghurt	Fermented	Dairy	Yoghurt	Bacteria	31.93
Unknown yoghurt	Fermented	Dairy	Yoghurt	Bacteria	3.15
Marmite	Fermented	Other	Marmite	Yeast	0.02
Dressing of oil/vinegar	Fermented	Other	Vinegar	Bacteria/yeast	1.34
Sandwich sausage	Unclear if sausage is fermented.	NA	NA	NA	3.05

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]					
Food[b]	**Fermentation Status**	**Food Group**	**Subgroup**	**(Main) Fermentation Organism**	**Mean intakes (g/day)**
Chocolate spread	Composite dish/fermented ingredient.	NA	NA	NA	4.92
Breakfast drink	Composite dish/fermented ingredient.	NA	NA	NA	3.28
Mousse (dessert)	Composite dish/fermented ingredient.	NA	NA	NA	5.28
Pizza	Composite dish/fermented ingredient.	NA	NA	NA	12.37
Dressing of light mayonnaise	Composite dish/fermented ingredient.	NA	NA	NA	1.68
Dressing of mayonnaise	Composite dish/fermented ingredient.	NA	NA	NA	0.25
Mayonnaise	Composite dish/fermented ingredient.	NA	NA	NA	0.85
Light mayonnaise	Composite dish/fermented ingredient.	NA	NA	NA	1.19
Ketchup	Composite dish/fermented ingredient.	NA	NA	NA	1.44
Nutrition biscuit	Unclear if yeast is used in the cooking process.	NA	NA	NA	4.05
Mayonnaise with snack	Composite dish/fermented ingredient.	NA	NA	NA	0.16
Light mayonnaise with snack	Composite dish/fermented ingredient.	NA	NA	NA	0.17
Ketchup with snack	Composite dish/fermented ingredient.	NA	NA	NA	0.28
Eggnog	Composite dish/fermented ingredient.	NA	NA	NA	0.20
Strong drink	Unclear if this is an alcoholic drink that is produced by fermentation.	NA	NA	NA	4.01
Skim milk or buttermilk	Unclear category.	NA	NA	NA	2.08
Whole wheat cracker	Unclear if yeast is used in the cooking process.	NA	NA	NA	1.88
Liver sausage	Unclear if sausage is fermented.	NA	NA	NA	0.42
24-h Recall					
Beer, >7 vol% alcohol	Fermented	Beverages	Beer	Yeast	382.25
Beer, alcohol free <0.1 vol% alcohol	Fermented	Beverages	Beer	Yeast	341.49
Beer, brown	Fermented	Beverages	Beer	Yeast	388.57
Beer, low alcohol 0.1-1.2 vol% alcohol	Fermented	Beverages	Beer	Yeast	250.00
Beer, pilsner (pale lager)	Fermented	Beverages	Beer	Yeast	464.51
Beer with fruit flavour	Fermented	Beverages	Beer	Yeast	330.00
Cider	Fermented	Beverages	Cider	Yeast	211.67
Cappuccino, freshly made	Fermented	Beverages	Coffee	Endogenous	199.67
Coffee, prepared	Fermented	Beverages	Coffee	Endogenous	209.08
Coffee, cappuccino instant prepared	Fermented	Beverages	Coffee, instant	Endogenous	236.56
Coffee with milk from vending machine	Fermented	Beverages	Coffee, instant	Endogenous	302.27
Coffee with sugar and milk from vending machine	Fermented	Beverages	Coffee, instant	Endogenous	222.79
Madeira	Fermented	Beverages	Wine	Endogenous	35.67
Port wine	Fermented	Beverages	Wine	Endogenous	74.28
Sherry	Fermented	Beverages	Wine	Endogenous	81.66
Medicinal wine (pleegzusterbloedwijn)	Fermented	Beverages	Wine	Endogenous	75.00
Wine, red	Fermented	Beverages	Wine	Endogenous	151.68
Wine, rose	Fermented	Beverages	Wine	Endogenous	157.12
Wine, white dry	Fermented	Beverages	Wine	Endogenous	151.79
Wine, white sweet	Fermented	Beverages	Wine	Endogenous	129.42
Baguette, brown	Fermented	Cereals/grains	Bread, brown	Yeast	65.52
Bread (Blue Brand Goede Start), light brown	Fermented	Cereals/grains	Bread, brown	Yeast	70.00
Bread brown with pumpkin seeds	Fermented	Cereals/grains	Bread, brown	Yeast	78.57

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]

Food[b]	Fermentation Status	Food Group	Subgroup	(Main) Fermentation Organism	Mean intakes (g/day)
Bread brown with seeds	Fermented	Cereals/grains	Bread, brown	Yeast	71.27
Bread brown with sunflower seeds	Fermented	Cereals/grains	Bread, brown	Yeast	71.26
Bread brown wheat	Fermented	Cereals/grains	Bread, brown	Yeast	70.76
Bread brown/wholemeal with muesli	Fermented	Cereals/grains	Bread, brown	Yeast	51.89
Bread brown/wholemeal with pumpkin seeds	Fermented	Cereals/grains	Bread, brown	Yeast	61.76
Bread brown/wholemeal with sunflower seeds	Fermented	Cereals/grains	Bread, brown	Yeast	73.04
Roll brown, hard	Fermented	Cereals/grains	Bread, brown	Yeast	68.01
Roll brown, soft	Fermented	Cereals/grains	Bread, brown	Yeast	76.25
Baguette with cheese-onion	Fermented	Cereals/grains	Bread, other	Yeast	79.00
Bread, ciabatta, no filling	Fermented	Cereals/grains	Bread, other	Yeast	87.65
Bread, currant	Fermented	Cereals/grains	Bread, other	Yeast	49.83
Bread, currant with almond paste	Fermented	Cereals/grains	Bread, other	Yeast	47.03
Bread, linseed	Fermented	Cereals/grains	Bread, other	Yeast	48.25
Bread loaf, gluten-free, Glutafin	Fermented	Cereals/grains	Bread, other	Yeast	78.11
Bread, low in carbohydrates	Fermented	Cereals/grains	Bread, other	Yeast	53.45
Bread, raisin	Fermented	Cereals/grains	Bread, other	Yeast	52.47
Bread, raisin/current average	Fermented	Cereals/grains	Bread, other	Yeast	48.81
Bread ,wheat malt Tarvo	Fermented	Cereals/grains	Bread, other	Yeast	79.80
Bun, currant/raisin	Fermented	Cereals/grains	Bread, other	Yeast	58.21
Bun with vanilla custard	Fermented	Cereals/grains	Bread, other	Yeast	76.67
Coconut bread, sweetened, sliced	Fermented	Cereals/grains	Bread, other	Yeast	10.71
Stollen with almond/paste, average	Fermented	Cereals/grains	Bread, other	Yeast	73.31
Stollen with almond/paste with nuts	Fermented	Cereals/grains	Bread, other	Yeast	62.56
Stollen with almond/paste without nuts	Fermented	Cereals/grains	Bread, other	Yeast	56.00
Bread, rye, average	Fermented	Cereals/grains	Bread, rye	Yeast	53.37
Bread, rye, dark	Fermented	Cereals/grains	Bread, rye	Yeast	46.31
Bread, rye, light	Fermented	Cereals/grains	Bread, rye	Yeast	72.77
Bread crumbs	Fermented	Cereals/grains	Bread, toasted	Yeast	9.11
Crackers cream	Fermented	Cereals/grains	Bread, toasted	Yeast	15.08
Crackers cream, low sodium	Fermented	Cereals/grains	Bread, toasted	Yeast	26.22
Crispbakes, Dutch	Fermented	Cereals/grains	Bread, toasted	Yeast	12.19
Crispbakes, Dutch wholemeal	Fermented	Cereals/grains	Bread, toasted	Yeast	13.42
Crispbread, average	Fermented	Cereals/grains	Bread, toasted	Yeast	18.05
Crispbread, Cracottes	Fermented	Cereals/grains	Bread, toasted	Yeast	13.08
Crispbread, Cracottes Vital	Fermented	Cereals/grains	Bread, toasted	Yeast	22.50
Crispbread, gold-brown	Fermented	Cereals/grains	Bread, toasted	Yeast	17.74
Crispbread, high fibre	Fermented	Cereals/grains	Bread, toasted	Yeast	17.63
Crispbread, light	Fermented	Cereals/grains	Bread, toasted	Yeast	9.35
Crispbread, poppy seeds	Fermented	Cereals/grains	Bread, toasted	Yeast	30.00
Crispbread, sandwich Wasa	Fermented	Cereals/grains	Bread, toasted	Yeast	31.67
Crispbread, sesame	Fermented	Cereals/grains	Bread, toasted	Yeast	20.05
Crispbread, wholemeal	Fermented	Cereals/grains	Bread, toasted	Yeast	21.10
Crispbread, wholemeal Cracottes	Fermented	Cereals/grains	Bread, toasted	Yeast	24.71

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]					
Food[b]	**Fermentation Status**	**Food Group**	**Subgroup**	**(Main) Fermentation Organism**	**Mean intakes (g/day)**
Pretzel sticks	Fermented	Cereals/grains	Bread, toasted	Yeast	18.76
Toast	Fermented	Cereals/grains	Bread, toasted	Yeast	12.19
Toast, Melba natural	Fermented	Cereals/grains	Bread, toasted	Yeast	10.72
Toast, Melba other varieties	Fermented	Cereals/grains	Bread, toasted	Yeast	21.08
Baguette, white	Fermented	Cereals/grains	Bread, white	Yeast	68.90
Bread, Blue Band Goede Start white bread	Fermented	Cereals/grains	Bread, white	Yeast	34.17
Bread, pita white	Fermented	Cereals/grains	Bread, white	Yeast	89.48
Bread, Tijger white	Fermented	Cereals/grains	Bread, white	Yeast	73.16
Bread, white average milk/water based	Fermented	Cereals/grains	Bread, white	Yeast	57.64
Bread, white milk based	Fermented	Cereals/grains	Bread, white	Yeast	58.29
Bread, white Turkish	Fermented	Cereals/grains	Bread, white	Yeast	99.50
Bread, white with sugar Suikerbrood	Fermented	Cereals/grains	Bread, white	Yeast	57.81
Bread, white with sunflower seeds	Fermented	Cereals/grains	Bread, white	Yeast	74.25
Bread, white, water based	Fermented	Cereals/grains	Bread, white	Yeast	62.67
Roll, white, hard	Fermented	Cereals/grains	Bread, white	Yeast	76.06
Roll, white, soft	Fermented	Cereals/grains	Bread, white	Yeast	62.19
Bread, current, wholemeal	Fermented	Cereals/grains	Bread, wholegrain	Yeast	53.81
Bread, multigrain, average with seeds	Fermented	Cereals/grains	Bread, wholegrain	Yeast	72.70
Bread, multigrain flour with seeds	Fermented	Cereals/grains	Bread, wholegrain	Yeast	91.33
Bread, multigrain, wholemeal with Becel	Fermented	Cereals/grains	Bread, wholegrain	Yeast	67.63
Bread, multigrain, wholemeal with seeds	Fermented	Cereals/grains	Bread, wholegrain	Yeast	73.21
Bread, sourdough, wholemeal	Fermented	Cereals/grains	Bread, wholegrain	Yeast/bacteria	81.48
Bread, Tijger brown, wheat	Fermented	Cereals/grains	Bread, wholegrain	Yeast	85.37
Bread, wheat rye, wholemeal	Fermented	Cereals/grains	Bread, wholegrain	Yeast	70.00
Bread, wholemeal, average	Fermented	Cereals/grains	Bread, wholegrain	Yeast	70.38
Bread, wholemeal, fine	Fermented	Cereals/grains	Bread, wholegrain	Yeast	74.45
Bread, wholemeal with nuts	Fermented	Cereals/grains	Bread, wholegrain	Yeast	61.96
Bread, wholemeal with pumpkin seeds	Fermented	Cereals/grains	Bread, wholegrain	Yeast	66.67
Bread wholemeal with seeds	Fermented	Cereals/grains	Bread, wholegrain	Yeast	74.28
Bread wholemeal with sunflower seeds	Fermented	Cereals/grains	Bread, wholegrain	Yeast	79.31
Bread wholemeal with wheat kernels	Fermented	Cereals/grains	Bread, wholegrain	Yeast	74.22
Bun wholemeal with muesli	Fermented	Cereals/grains	Bread, wholegrain	Yeast	67.67
Roll, multigrain, hard	Fermented	Cereals/grains	Bread, wholegrain	Yeast	72.71
Roll, multigrain, soft	Fermented	Cereals/grains	Bread, wholegrain	Yeast	56.73
Roll, wholemeal, soft	Fermented	Cereals/grains	Bread, wholegrain	Yeast	72.14
Dough for pizza and savoury pie	Fermented	Cereals/grains	Other	Yeast	91.93
Doughnut, Dutch style	Fermented	Cereals/grains	Other	Yeast	68.08
Doughnuts, plain	Fermented	Cereals/grains	Other	Yeast	49.00
Almond filled pastry	Fermented	Cereals/grains	Pastry	Yeast	47.81
Croissant, average	Fermented	Cereals/grains	Pastry	Yeast	41.69
Croissant, chocolate	Fermented	Cereals/grains	Pastry	Yeast	50.33
Croissant prepared with butter	Fermented	Cereals/grains	Pastry	Yeast	45.50
Croissant prepared without butter	Fermented	Cereals/grains	Pastry	Yeast	45.00

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]

Food[b]	Fermentation Status	Food Group	Subgroup	(Main) Fermentation Organism	Mean intakes (g/day)
Croissant with ham and cheese	Fermented	Cereals/grains	Pastry	Yeast	65.25
Croissant with cheese	Fermented	Cereals/grains	Pastry	Yeast	44.29
Croissants	Fermented	Cereals/grains	Pastry	Yeast	47.61
Danish pastry	Fermented	Cereals/grains	Pastry	Yeast	62.95
Waffle (Luikse)	Fermented	Cereals/grains	Pastry	Yeast	55.00
Waffle, soft-/sugar-/flash-	Fermented	Cereals/grains	Pastry	Yeast	43.25
Chocolate bar milk with nuts	Fermented	Cocoa	Chocolate	Endogenous	28.32
Chocolate bar milk without sugar	Fermented	Cocoa	Chocolate	Endogenous	6.20
Chocolate, confetti, average	Fermented	Cocoa	Chocolate	Endogenous	14.10
Chocolate, confetti, milk	Fermented	Cocoa	Chocolate	Endogenous	15.01
Chocolate, confetti, mix of white and plain	Fermented	Cocoa	Chocolate	Endogenous	14.25
Chocolate, confetti, plain	Fermented	Cocoa	Chocolate	Endogenous	15.52
Chocolate, confetti, white	Fermented	Cocoa	Chocolate	Endogenous	11.33
Chocolate flakes, average	Fermented	Cocoa	Chocolate	Endogenous	14.33
Chocolate flakes, milk	Fermented	Cocoa	Chocolate	Endogenous	13.69
Chocolate flakes, plain	Fermented	Cocoa	Chocolate	Endogenous	16.23
Chocolate liqueurs	Fermented	Cocoa	Chocolate	Endogenous	15.15
Chocolate, plain	Fermented	Cocoa	Chocolate	Endogenous	15.24
Chocolate, plain with nuts	Fermented	Cocoa	Chocolate	Endogenous	20.00
Chocolate, plain without sugar	Fermented	Cocoa	Chocolate	Endogenous	10.67
Chocolate white	Fermented	Cocoa	Chocolate	Endogenous	17.96
Chocolates, filled/Belguim chocolate	Fermented	Cocoa	Chocolate	Endogenous	21.72
Milk chocolate	Fermented	Cocoa	Chocolate	Endogenous	18.33
Milk chocolate with hazelnuts wo sugar	Fermented	Cocoa	Chocolate	Endogenous	48.83
Milk chocolate with puffed rice	Fermented	Cocoa	Chocolate	Endogenous	16.50
Cocoa powder	Fermented	Cocoa	Chocolate, powder	Endogenous	2.33
Buttermilk	Fermented	Dairy	Buttermilk	Bacteria	211.36
Buttermilk with fruit	Fermented	Dairy	Buttermilk	Bacteria	207.86
Cheese 10+	Fermented	Dairy	Cheese, hard	Bacteria	36.00
Cheese 20+	Fermented	Dairy	Cheese, hard	Bacteria	20.96
Cheese 20+, Leiden with cumin/Frisian clove	Fermented	Dairy	Cheese, hard	Bacteria	32.00
Cheese 20+, rindless	Fermented	Dairy	Cheese, hard	Bacteria	20.00
Cheese 30+	Fermented	Dairy	Cheese, hard	Bacteria	24.45
Cheese 30+, low salt	Fermented	Dairy	Cheese, hard	Bacteria	21.53
Cheese 40+, Leiden with cumin/Frisian clove	Fermented	Dairy	Cheese, hard	Bacteria	27.63
Cheese 40+, sodium reduced	Fermented	Dairy	Cheese, hard	Bacteria	20.67
Cheese 45+	Fermented	Dairy	Cheese, hard	Bacteria	27.68
Cheese 48+, low salt	Fermented	Dairy	Cheese, hard	Bacteria	22.80
Cheese 50+	Fermented	Dairy	Cheese, hard	Bacteria	24.36
Cheese, Amsterdam 48+	Fermented	Dairy	Cheese, hard	Bacteria	22.94
Cheese, Bluefort	Fermented	Dairy	Cheese, hard	Bacteria	33.14
Cheese, Cheddar	Fermented	Dairy	Cheese, hard	Bacteria	23.50
Cheese, Edam 40+	Fermented	Dairy	Cheese, hard	Bacteria	26.31

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]					
Food[b]	**Fermentation Status**	**Food Group**	**Subgroup**	**(Main) Fermentation Organism**	**Mean intakes (g/day)**
Cheese, Emmenthaler	Fermented	Dairy	Cheese, hard	Bacteria	29.02
Cheese, goat, hard	Fermented	Dairy	Cheese, hard	Bacteria	25.42
Cheese, Gouda 48+, aged 10-12 mths	Fermented	Dairy	Cheese, hard	Bacteria	27.63
Cheese, Gouda 48+, aged 4-7 mths	Fermented	Dairy	Cheese, hard	Bacteria	24.71
Cheese, Gouda 48+, aged 4-8 weeks	Fermented	Dairy	Cheese, hard	Bacteria	25.98
Cheese, Gouda 48+, aged 8 wk-4 mths	Fermented	Dairy	Cheese, hard	Bacteria	25.16
Cheese, Gouda 48+, average	Fermented	Dairy	Cheese, hard	Bacteria	25.49
Cheese, Gruyere	Fermented	Dairy	Cheese, hard	Bacteria	31.60
Cheese, Parmesan	Fermented	Dairy	Cheese, hard	Bacteria	19.17
Cheese, processed, rindless 40+	Fermented	Dairy	Cheese, hard	Bacteria	25.06
Cheese product with vegetable fat 45+	Fermented	Dairy	Cheese, hard	Bacteria	17.00
Cheese, raw milk 48+	Fermented	Dairy	Cheese, hard	Bacteria	28.17
Cheese, smoked	Fermented	Dairy	Cheese, hard	Bacteria	20.57
Cheese, Swiss, dried	Fermented	Dairy	Cheese, hard	Bacteria	6.29
Cheese, Gorgonzola	Fermented	Dairy	Cheese, medium	Bacteria/mold	43.16
Cheese, Leerdammer/Maasdammer 45+	Fermented	Dairy	Cheese, medium	Bacteria	32.87
Cheese, Roquefort	Fermented	Dairy	Cheese, medium	Bacteria/mold	27.63
Cheese, Saint Paulin/Port Salut	Fermented	Dairy	Cheese, medium	Bacteria	40.80
Cheese, Stilton	Fermented	Dairy	Cheese, medium	Bacteria/mold	28.67
Cheese, Brie 50+	Fermented	Dairy	Cheese, soft	Bacteria/mold	22.00
Cheese, Brie 60+	Fermented	Dairy	Cheese, soft	Bacteria/mold	30.58
Cheese, Camembert 30+	Fermented	Dairy	Cheese, soft	Bacteria/mold	12.94
Cheese, Camembert 45+	Fermented	Dairy	Cheese, soft	Bacteria/mold	53.16
Cheese, goat fresh	Fermented	Dairy	Cheese, soft	Bacteria	33.67
Cheese, Kernhem 60+	Fermented	Dairy	Cheese, soft	Bacteria	38.50
Cheese, Rambol	Fermented	Dairy	Cheese, soft	Bacteria	21.33
Cheese, sheep fresh	Fermented	Dairy	Cheese, soft	Bacteria	36.07
Cream, sour	Fermented	Dairy	Cream	Bacteria	24.50
Creme fraiche	Fermented	Dairy	Cream	Bacteria	24.34
Creme fraiche, half fat	Fermented	Dairy	Cream	Bacteria	51.33
Fromage frais, full fat	Fermented	Dairy	Quark	Bacteria	109.81
Fromage frais, full fat with fruit	Fermented	Dairy	Quark	Bacteria	129.00
Fromage frais, half fat	Fermented	Dairy	Quark	Bacteria	99.92
Fromage frais, half fat with fruit	Fermented	Dairy	Quark	Bacteria	121.30
Fromage frais, low fat	Fermented	Dairy	Quark	Bacteria	122.80
Fromage frais, low fat with fruit with sweetener	Fermented	Dairy	Quark	Bacteria	180.96
Fromage frais, low fat with fruit	Fermented	Dairy	Quark	Bacteria	130.00
Fromage frais, with fruit Danoontje	Fermented	Dairy	Quark	Bacteria	85.71
Fromage frais, yoghurt with fruit	Fermented	Dairy	Quark	Bacteria	144.84
Whey drink, light Rivella	Fermented	Dairy	Whey drink	NA	194.55
Whey drink, Taksi with sugar	Fermented	Dairy	Whey drink	NA	185.42
Whey drink, Taksi with sugar and sweetener	Fermented	Dairy	Whey drink	NA	223.33
Whey drink, Taksi with sweetener	Fermented	Dairy	Whey drink	NA	175.00

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]

Food[b]	Fermentation Status	Food Group	Subgroup	(Main) Fermentation Organism	Mean intakes (g/day)
Yakult	Fermented	Dairy	Yakult	Bacteria	67.27
Yakult light	Fermented	Dairy	Yakult	Bacteria	65.00
Yoghurt, 0% fat with fruit (Activia)	Fermented	Dairy	Yoghurt	Bacteria	135.58
Yoghurt, creamy- with fruit	Fermented	Dairy	Yoghurt	Bacteria	118.00
Yoghurt, full fat	Fermented	Dairy	Yoghurt	Bacteria	137.26
Yoghurt, full fat, natural (Activia)	Fermented	Dairy	Yoghurt	Bacteria	143.75
Yoghurt, full fat with fruit/muesli (Activia)	Fermented	Dairy	Yoghurt	Bacteria	125.14
Yoghurt, full fat with fruit	Fermented	Dairy	Yoghurt	Bacteria	143.14
Yoghurt, Greek, full fat	Fermented	Dairy	Yoghurt	Bacteria	113.26
Yoghurt, half fat	Fermented	Dairy	Yoghurt	Bacteria	161.36
Yoghurt, half fat (Vifit naturel)	Fermented	Dairy	Yoghurt	Bacteria	183.33
Yoghurt, half fat with fruit	Fermented	Dairy	Yoghurt	Bacteria	138.41
Yoghurt, low fat	Fermented	Dairy	Yoghurt	Bacteria	154.21
Yoghurt, low fat (Bulgarian)	Fermented	Dairy	Yoghurt	Bacteria	7.62
Yoghurt, low fat with fruit with sw Vitalinea	Fermented	Dairy	Yoghurt	Bacteria	164.29
Yoghurt, low fat with fruit/van with sweetener (Optimel)	Fermented	Dairy	Yoghurt	Bacteria	174.19
Yoghurt, low fat with fruit	Fermented	Dairy	Yoghurt	Bacteria	142.54
Yoghurt, low fat with fruit with sweeteners	Fermented	Dairy	Yoghurt	Bacteria	139.67
Yoghurt, vanilla, half fat	Fermented	Dairy	Yoghurt	Bacteria	144.37
Yoghurt, whole milk (Bulgarian)	Fermented	Dairy	Yoghurt	Bacteria	66.54
Actimel	Fermented	Dairy	Yoghurt, drink product	Bacteria	99.22
Actimel light	Fermented	Dairy	Yoghurt, drink product	Bacteria	112.50
Breakfast drink (Breaker Breakfast)	Fermented	Dairy	Yoghurt, drink product	Bacteria	200.00
Yoghurt drink	Fermented	Dairy	Yoghurt, drink product	Bacteria	177.42
Yoghurt drink (Becel pro-activ)	Fermented	Dairy	Yoghurt, drink product	Bacteria	112.17
Yoghurt drink (Fristi)	Fermented	Dairy	Yoghurt, drink product	Bacteria	198.75
Yoghurt drink (Fristi) with sweetners	Fermented	Dairy	Yoghurt, drink product	Bacteria	130.00
Yoghurt drink (Vifit), fruit	Fermented	Dairy	Yoghurt, drink product	Bacteria	230.60
Yoghurt drink (Vifit), fruit, light	Fermented	Dairy	Yoghurt, drink product	Bacteria	249.29
Yoghurt drink (Vifit), natural	Fermented	Dairy	Yoghurt, drink product	Bacteria	175.00
Yoghurt drink with sweeteners (Optimel)	Fermented	Dairy	Yoghurt, drink product	Bacteria	207.83
Yoghurt drink with sweetners	Fermented	Dairy	Yoghurt, drink product	Bacteria	181.25
Yoghurt drink (Yomild), fruit	Fermented	Dairy	Yoghurt, drink product	Bacteria	222.50
Yoghurt drink (Yomild), naturel	Fermented	Dairy	Yoghurt, drink product	Bacteria	156.40
Yoghurt product (Becel pro-activ)	Fermented	Dairy	Yoghurt, drink product	Bacteria	166.67
Yoghurt snack (Breaker)	Fermented	Dairy	Yoghurt, drink product	Bacteria	200.00
Vegetables, mixed pickled (Atjar tjampoer)	Fermented	Fruits/Vegetables	Other	Bacteria	68.42
Cabbage, sauerkraut, cooked	Fermented	Fruits/Vegetables	Sauerkraut	Bacteria	141.94
Cabbage, sauerkraut, raw	Fermented	Fruits/Vegetables	Sauerkraut	Bacteria	60.91
Juice, sauerkraut	Fermented	Fruits/Vegetables	Sauerkraut	Bacteria	220.00
Herring, salted	Fermented	Meat/Fish	Fish	Endogenous	101.66
Salami	Fermented	Meat/Fish	Sausages	Bacteria	24.82

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]					
Food[b]	**Fermentation Status**	**Food Group**	**Subgroup**	**(Main) Fermentation Organism**	**Mean intakes (g/day)**
Salami sausage, saveloy	Fermented	Meat/Fish	Sausages	Bacteria	23.81
Salami sausage, saveloy, lean	Fermented	Meat/Fish	Sausages	Bacteria	32.60
Sausage, Chorizo	Fermented	Meat/Fish	Sausages	Bacteria	32.31
Sausage, dry salami, Turkish	Fermented	Meat/Fish	Sausages	Bacteria	33.75
Yeast extract, Marmite	Fermented	Other	Marmite	Yeast	3.56
Vinegar	Fermented	Other	Vinegar	Bacteria/yeast	8.30
Tempeh, fermented soya beans	Fermented	Soya	Soya	Mold/Bacteria	49.60
Soya sauce, savoury	Fermented	Soya	Soya sauce	Mold/Bacteria/Yeast	14.05
Soya sauce, sweet	Fermented	Soya	Soya sauce	Mold/Bacteria/Yeast	13.98
Soya sauce, sweet, low sodium	Fermented	Soya	Soya sauce	Mold/Bacteria/Yeast	7.00
Kale curly with mashed potatoes and sausage	Composite dish/fermented ingredient.	NA	NA	NA	385.00
Fried rice, Nasi goring, frozen/tinned	Composite dish/fermented ingredient.	NA	NA	NA	350.00
Sauerkraut with mashed potatoes without meat	Composite dish/fermented ingredient.	NA	NA	NA	342.44
Lasagna with meat and sauce, frozen	Composite dish/fermented ingredient.	NA	NA	NA	333.33
Lasagna with meat and sauce, ready-to-eat	Composite dish/fermented ingredient.	NA	NA	NA	292.27
Spaghetti Bolognese, frozen	Composite dish/fermented ingredient.	NA	NA	NA	259.00
Porridge, buttermilk with wheat flour	Composite dish/fermented ingredient.	NA	NA	NA	255.00
Chinese noodle dish, Bami goreng frozen	Composite dish/fermented ingredient.	NA	NA	NA	253.33
Fried rice, Nasi goreng, ready-to-eat	Composite dish/fermented ingredient.	NA	NA	NA	235.38
Pizza, salami, frozen, unprepared	Composite dish/fermented ingredient.	NA	NA	NA	227.19
Pizza with ham and pineapple, frozen, unprepared	Composite dish/fermented ingredient.	NA	NA	NA	223.79
Chinese noodles, Bami goreng, ready-to-eat	Composite dish/fermented ingredient.	NA	NA	NA	223.06
Pizza with meat frozen, unprepared	Composite dish/fermented ingredient.	NA	NA	NA	220.52
Quiche with vegetables/cheese/egg	Composite dish/fermented ingredient.	NA	NA	NA	219.19
Pizza with cheese and vegetables, frozen	Composite dish/fermented ingredient.	NA	NA	NA	216.86
Smoothie, fruit with dairy	Composite dish/fermented ingredient.	NA	NA	NA	213.33
Pizza with fish, frozen, unprepared	Composite dish/fermented ingredient.	NA	NA	NA	209.50
Fried rice Nasi goreng with egg	Composite dish/fermented ingredient.	NA	NA	NA	203.09
Spinach, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	200.00
Pizza with frozen meat	Composite dish/fermented ingredient.	NA	NA	NA	195.17
Chinese noodle, dish Bami goreng, without egg	Composite dish/fermented ingredient.	NA	NA	NA	186.59
Pizza with cheese and tomato	Composite dish/fermented ingredient.	NA	NA	NA	181.23
Pizza with frozen vegetables	Composite dish/fermented ingredient.	NA	NA	NA	175.66
Beans, baked in tomato sauce, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	170.83
Spinach, creamed, frozen, boiled	Composite dish/fermented ingredient.	NA	NA	NA	167.15
Hot chocolate from vending machine	Composite dish/fermented ingredient.	NA	NA	NA	165.37
Peas, marrowfat legumes, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	164.82
Pancake	Composite dish/fermented ingredient.	NA	NA	NA	161.93
Tortellini, boiled	Composite dish/fermented ingredient.	NA	NA	NA	158.00
Peas and carrots, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	156.53
Peas, garden extra fine, low sodium, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	150.00
Porridge, buttermilk groats	Composite dish/fermented ingredient.	NA	NA	NA	148.33

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]

Food[b]	Fermentation Status	Food Group	Subgroup	(Main) Fermentation Organism	Mean intakes (g/day)
Tart with bread base with veg/egg/cheese	Composite dish/fermented ingredient.	NA	NA	NA	141.08
Carrots, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	135.92
Ragout with meat, all types	Composite dish/fermented ingredient.	NA	NA	NA	132.00
Beans, brown, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	130.72
Pizza with cross-bottom, frozen	Composite dish/fermented ingredient.	NA	NA	NA	125.00
Mackerel in oil, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	125.00
Beetroot, pickled, glass	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	123.75
Dessert made of custard, yoghurt & syrup	Composite dish/fermented ingredient.	NA	NA	NA	121.78
Tomatoes, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	121.52
Pork, Chinese style, Babi pangang, without rice	Composite dish/fermented ingredient.	NA	NA	NA	121.03
Cabbage, red, glass	Processed/tinned but unclear if fermented.	NA	NA	NA	120.55
Macaroni with ham & cheese sauce, tinned	Composite dish/fermented ingredient.	NA	NA	NA	120.00
Strawberry puree, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	120.00
Beans, French, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	117.06
Peas, garden, medium fine, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	116.84
Croquettes, potato, frozen, unprepared	Composite dish/fermented ingredient.	NA	NA	NA	115.75
Pears in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	113.39
Herring fillet in tomato sauce, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	110.67
Herring, smoked	Processed/tinned but unclear if fermented.	NA	NA	NA	110.00
Cheesecake made with fromage frais	Composite dish/fermented ingredient.	NA	NA	NA	108.66
Pea, garden super fine, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	108.00
Beans, broad, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	104.00
Raisins coated with milk chocolate	Composite dish/fermented ingredient.	NA	NA	NA	100.67
Apple sauce, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	99.93
Chocolate pudding with sauce	Composite dish/fermented ingredient.	NA	NA	NA	98.33
Salad, Russian	Composite dish/fermented ingredient.	NA	NA	NA	89.99
Chop-suey, Indonesian, without rice	Composite dish/fermented ingredient.	NA	NA	NA	89.93
Tangerines in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	89.54
Herring, pickled with gherkin	Composite dish/fermented ingredient.	NA	NA	NA	87.33
Snack sausage roll with bread, dough pastry	Composite dish/fermented ingredient.	NA	NA	NA	84.00
Peaches in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	83.72
Apricots in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	80.00
Plums in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	80.00
Apple sauce without sugar, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	78.16
Herring, pickled (sweet and sour)	Composite dish/fermented ingredient.	NA	NA	NA	77.85
Fruit cocktail in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	76.58
Foe jung hai, filled omelette	Composite dish/fermented ingredient.	NA	NA	NA	73.84
Salmon, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	72.69
Mussels, pickled	Composite dish/fermented ingredient.	NA	NA	NA	72.33
Gammon, boiled, deboned	Processed/tinned but unclear if fermented.	NA	NA	NA	71.81
Sauce, warm liquid, ready-made (<12% fat)	Composite dish/fermented ingredient.	NA	NA	NA	71.62
Chocolate eclair	Composite dish/fermented ingredient.	NA	NA	NA	70.00

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]					
Food[b]	**Fermentation Status**	**Food Group**	**Subgroup**	**(Main) Fermentation Organism**	**Mean intakes (g/day)**
Mushrooms, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	69.75
Cucumber, sliced, pickled	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	68.50
Tuna in oil, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	68.15
Sardines/pilchards in oil, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	66.75
Mackerel in water, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	65.96
Flan filling, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	65.00
Asparagus, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	63.50
Teacakes, chocolate-coated marshmellow	Composite dish/fermented ingredient.	NA	NA	NA	63.00
Roti Surinam pancake	Composite dish/fermented ingredient.	NA	NA	NA	62.00
Tuna in water, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	61.82
Cherries in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	59.56
Snack sausage roll with bread dough frozen	Composite dish/fermented ingredient.	NA	NA	NA	56.50
Sauce, cheese	Processed cheese.	NA	NA	NA	56.04
Biscuits, fortified (Fruitkick Extra)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	54.00
Advocaat liqueur	Composite dish/fermented ingredient.	NA	NA	NA	53.41
Biscuits, spiced Speculaas with almond paste	Unclear if yeast or another leavening agent is used.	NA	NA	NA	51.46
Sauce, warm liquid, ready-made (>12% fat)	Composite dish/fermented ingredient.	NA	NA	NA	50.50
Processed meat product, low sodium, average	Unclear if processing is fermentation or another method.	NA	NA	NA	50.00
Sauce, oriental, ready-made in jar/bag	Composite dish/fermented ingredient.	NA	NA	NA	47.67
Pineapple in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	47.58
Cake wrapped in marzipan and chocolate	Composite dish/fermented ingredient.	NA	NA	NA	46.23
Luncheon meat, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	46.00
Muesli, crunchy with chocolate	Composite dish/fermented ingredient.	NA	NA	NA	44.80
Eel, smoked	Processed/tinned but unclear if fermented.	NA	NA	NA	44.00
Sweet pepper, sweet/sour, pickled	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	43.55
Pastry puff cheese, filled, unprepared	Composite dish/fermented ingredient.	NA	NA	NA	43.54
Mango chutney	Composite dish/fermented ingredient.	NA	NA	NA	42.00
Biscuits, oatmeal	Unclear if yeast or another leavening agent is used.	NA	NA	NA	41.17
Fruit in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	40.07
Biscuits fortified with currants (LigaEvergreen)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	38.63
Sauce Chinese, sweet & sour, home-made preparation	Composite dish/fermented ingredient.	NA	NA	NA	38.34
Biscuits, Jaffa cakes/Cake (PiM's)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	38.00
Cake, chocolate made with butter	Composite dish/fermented ingredient.	NA	NA	NA	37.88
Biscuits, fortified (Liga Milkbreak)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	37.50
M&M's, chocolate with peanuts	Composite dish/fermented ingredient.	NA	NA	NA	37.43
Raspberry/red currant puree, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	36.67
Sausage excluding liver, average	Unclear if fermented.	NA	NA	NA	34.89
Bamboo shoots, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	34.63
Biscuits, fruit	Unclear if yeast or another leavening agent is used.	NA	NA	NA	33.67
Candybar, KitKat	Composite dish/fermented ingredient.	NA	NA	NA	33.35

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]

Food[b]	Fermentation Status	Food Group	Subgroup	(Main) Fermentation Organism	Mean intakes (g/day)
Biscuits, fortified (Liga Evergreen? assortment)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	31.92
Tomatoes dried in oil tin/glass	Processed/tinned but unclear if fermented.	NA	NA	NA	31.90
Candybar, Twix	Composite dish/fermented ingredient.	NA	NA	NA	31.73
Sausage raw beef (ossenworst)	Unclear if fermented.	NA	NA	NA	31.33
Candybar, Snickers	Composite dish/fermented ingredient.	NA	NA	NA	30.62
Gherkins, sweet, pickled	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	30.28
Candybar, Bounty	Composite dish/fermented ingredient.	NA	NA	NA	29.67
Cake made with butter	Composite dish/fermented ingredient.	NA	NA	NA	29.63
Cake, butter, Dutch (Boterkoek)	Composite dish/fermented ingredient.	NA	NA	NA	29.42
Sauce, barbecue	Composite dish/fermented ingredient.	NA	NA	NA	29.41
Eclair, cheese cream filled	Composite dish/fermented ingredient.	NA	NA	NA	29.33
Sauce (Joppie)	Composite dish/fermented ingredient.	NA	NA	NA	28.75
Herb paste (boemboe)	Composite dish/fermented ingredient.	NA	NA	NA	27.74
Biscuits, chocolate-coated (Chocoprins)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	27.16
Beef, salted, cooked	Processed/tinned but unclear if fermented.	NA	NA	NA	27.00
Sauce for chips (35% oil)	Composite dish/fermented ingredient.	NA	NA	NA	26.73
Biscuits with dried fruit & yoghurt (Yofruit)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	25.89
Tomato puree, concentrated, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	25.85
Biscuits, filled (Prince)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	25.75
Biscuits & snacks, cheesy, average	Unclear if yeast or another leavening agent is used.	NA	NA	NA	25.36
Bacon rashers, streaky	Processed/tinned but unclear if fermented.	NA	NA	NA	25.18
Ketchup, curry	Composite dish/fermented ingredient.	NA	NA	NA	25.16
Onions, silverskin, sweet, pickled without sugar	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	25.00
Syrup from tinned fruit	Processed/tinned but unclear if fermented.	NA	NA	NA	24.75
Gherkins, sour, pickled	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	24.69
Cheese spread 60+ (Kiri)	Processed cheese.	NA	NA	NA	24.50
Ketchup, tomato	Composite dish/fermented ingredient.	NA	NA	NA	24.38
Sauce, cocktail/party/table (>25% oil)	Composite dish/fermented ingredient.	NA	NA	NA	23.93
Biscuits, fortified (Liga Fruitkick)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	23.50
Olives, tinned/glass	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	23.44
Salad dressing (20% oil with yogurt)	Composite dish/fermented ingredient.	NA	NA	NA	23.38
Biscuits, salted, average	Unclear if yeast or another leavening agent is used.	NA	NA	NA	23.15
Cheese spread 45+	Processed cheese.	NA	NA	NA	23.00
Dairy spread, plain/herbs light	Processed cheese.	NA	NA	NA	22.98
Sausage including liver products, average	Unclear if fermented.	NA	NA	NA	22.80
Muesli bar with chocolate	Composite dish/fermented ingredient.	NA	NA	NA	22.80
Bilberries in syrup tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	22.33
Salad dressing/sauce, approximately 13% oil	Composite dish/fermented ingredient.	NA	NA	NA	22.26
Sauce, chocolate, for pudding	Composite dish/fermented ingredient.	NA	NA	NA	21.58
Liver paté	Processed/tinned but unclear if fermented.	NA	NA	NA	21.55

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]					
Food[b]	**Fermentation Status**	**Food Group**	**Subgroup**	**(Main) Fermentation Organism**	**Mean intakes (g/day)**
Cheese spread 40+	Processed cheese.	NA	NA	NA	21.50
Biscuits, Dutch, shortbread (spritsstukken)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	21.37
Salad cream (25% oil)	Composite dish/fermented ingredient.	NA	NA	NA	21.26
Biscuits, muesli	Unclear if yeast or another leavening agent is used.	NA	NA	NA	21.13
Salad dressing, honey/mustard	Composite dish/fermented ingredient.	NA	NA	NA	20.64
Paté, averag	Processed/tinned but unclear if fermented.	NA	NA	NA	20.50
Cheese spread 30+	Processed cheese.	NA	NA	NA	20.17
Sauce, cocktail/party/table (25% oil)	Composite dish/fermented ingredient.	NA	NA	NA	19.94
Mayonnaise, low fat (40% oil)	Composite dish/fermented ingredient.	NA	NA	NA	19.86
Biscuits, brown/wholemeal	Unclear if yeast or another leavening agent is used.	NA	NA	NA	19.66
Biscuits with chocolate layer (Scholiertje)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	19.50
Capers	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	18.85
Ham, smoked, raw	Unclear if fermented.	NA	NA	NA	18.81
Sauce for chips (25% oil)	Composite dish/fermented ingredient.	NA	NA	NA	18.77
Cocoa powder, sweetened (Nesquik Plus)	Composite dish/fermented ingredient.	NA	NA	NA	18.50
Biscuits, sugar free	Unclear if yeast or another leavening agent is used.	NA	NA	NA	18.43
Dairy spread, plain/herbs	Processed cheese.	NA	NA	NA	18.33
Chocolate chip cookie	Composite dish/fermented ingredient.	NA	NA	NA	18.31
Cheese spread 48+	Processed cheese.	NA	NA	NA	18.31
Spread, chocolate hazelnut	Composite dish/fermented ingredient.	NA	NA	NA	18.28
Corned beef	Processed/tinned but unclear if fermented.	NA	NA	NA	18.27
Cheese spread 20+	Processed cheese.	NA	NA	NA	18.10
Biscuits, chocolate	Unclear if yeast or another leavening agent is used.	NA	NA	NA	17.98
Sausage luncheon meat (boterham worst)	Unclear if fermented.	NA	NA	NA	17.70
M&M's chocolate	Composite dish/fermented ingredient.	NA	NA	NA	17.69
Tapenade olive	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	17.55
Salad dressing (40% oil) (Becel)	Composite dish/fermented ingredient.	NA	NA	NA	17.40
Mayonnaise, yoghurt-based (25% oil)	Composite dish/fermented ingredient.	NA	NA	NA	17.32
Mayonnaise	Composite dish/fermented ingredient.	NA	NA	NA	17.30
Biscuits, digestive with chocolate	Unclear if yeast or another leavening agent is used.	NA	NA	NA	17.00
Spread, chocolate, milk	Composite dish/fermented ingredient.	NA	NA	NA	16.94
Processed meat products, 10-20 g fat/100 g	Unclear if processing is fermentation or another method.	NA	NA	NA	16.93
Processed meat products, average	Processed/tinned but unclear if fermented.	NA	NA	NA	16.67
Biscuits, Dutch (krakeling)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	16.52
Biscuits, spiced (Speculaas)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	16.38
Melon in syrup, canned	Processed/tinned but unclear if fermented.	NA	NA	NA	16.00
Spread, chocolate, plain	Composite dish/fermented ingredient.	NA	NA	NA	15.95
Biscuits, shortbread, Bastogne	Unclear if yeast or another leavening agent is used.	NA	NA	NA	15.88
Salad dressing / vinaigrette	Composite dish/fermented ingredient.	NA	NA	NA	15.82
Biscuits, fortified (Liga Continue vitamin)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	15.75
Spread, chocolate (Duo Penotti hazelnut)	Composite dish/fermented ingredient.	NA	NA	NA	15.35

Table S1. Identification and classification of fermented foods from the FFQ and 24-h recalls in NQplus[a]

Food[b]	Fermentation Status	Food Group	Subgroup	(Main) Fermentation Organism	Mean intakes (g/day)
Beans, runner, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	15.00
Processed meat products (>30 g fat/ 100g)	Unclear if processing is fermentation or another method.	NA	NA	NA	15.00
Processed meat products (20-30 g fat/100g)	Unclear if processing is fermentation or another method.	NA	NA	NA	15.00
Syrup, malt barley	Fermentation ingredient but not fermented itself.	NA	NA	NA	15.00
Spread, duo with chocolate	Composite dish/fermented ingredient.	NA	NA	NA	14.79
Piccalilly (South Asian pickles product)	Composite dish/fermented ingredient.	NA	NA	NA	14.75
Forcemeat balls for soup, canned	Processed/tinned but unclear if fermented.	NA	NA	NA	14.50
Brawn, pork pickled in vinegar	Composite dish/fermented ingredient.	NA	NA	NA	14.35
Biscuits, sponge fingers	Unclear if yeast or another leavening agent is used.	NA	NA	NA	14.33
Cocoa powder, sweetened (Benco)	Composite dish/fermented ingredient.	NA	NA	NA	14.25
Candybar, Milky Way	Composite dish/fermented ingredient.	NA	NA	NA	14.20
Cocoa product, powder (Ovomaltine)	Composite dish/fermented ingredient.	NA	NA	NA	14.00
Maltesers	Composite dish/fermented ingredient.	NA	NA	NA	14.00
Biscuits, assorted with butter	Unclear if yeast or another leavening agent is used.	NA	NA	NA	13.85
Pork side, cured and smoked (casselerrib)	Unclear if fermented.	NA	NA	NA	13.83
Salad dressing, naturel, without oil	Composite dish/fermented ingredient.	NA	NA	NA	13.78
Biscuits, average	Unclear if yeast or another leavening agent is used.	NA	NA	NA	13.74
Anchovy in oil, canned	Processed/tinned but unclear if fermented.	NA	NA	NA	13.57
Toffee with chocolate	Composite dish/fermented ingredient.	NA	NA	NA	13.29
Crab in water, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	12.94
Chocolate spread, white	Composite dish/fermented ingredient.	NA	NA	NA	12.50
Mayonnaise product (approximately 35% oil)	Composite dish/fermented ingredient.	NA	NA	NA	12.20
Beef, smoke-dried	Unclear if fermented.	NA	NA	NA	12.18
Silver-skin onions, sweet, pickled, glass	Pickled (probably with vinegar) but unclear if fermented.	NA	NA	NA	11.92
Biscuits, sweet	Unclear if yeast or another leavening agent is used.	NA	NA	NA	11.68
After eight chocolate mints	Composite dish/fermented ingredient.	NA	NA	NA	11.25
Biscuits, gluten-free (Glutafin)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	11.00
Ginger stem in syrup, tinned	Processed/tinned but unclear if fermented.	NA	NA	NA	9.00
Ketchup, spicy	Composite dish/fermented ingredient.	NA	NA	NA	8.79
Biscuits, Dutch (Frou frou)	Unclear if yeast or another leavening agent is used.	NA	NA	NA	8.21
Mustard	Composite dish/fermented ingredient.	NA	NA	NA	7.43
Pepper, red hot paste	Composite dish/fermented ingredient.	NA	NA	NA	4.49
Pepper, red hot, fried paste	Composite dish/fermented ingredient.	NA	NA	NA	2.00

NA = not applicable

[a] Ordered by fermentation status, food group, then subgroup. The following items are included in this table: foods with a fermentation status of "fermented", composite dishes containing a fermented ingredient, and foods with unclear fermentation status. Other food items in the FFQ or 24-h recall that were clearly not fermented are not included in this table.

[b] Cheeses could be classified as skim, semi-skim, and full-fat based on the guidelines set by the Dutch Dairy Commodities Act (Overheid.nl. Warenwetbesluit Zuivel). Cheeses with designations of 45+ to 60+ were considered to be full-fat, 10+ to 40+ semi-skim, and ≤10 skim.

Table S2. Mean individual percent difference and test statistics from Bland-Altman analyses for the validation sample (n = 809)[a]

Foods and Food Groups	%Difference in individual means[b]	Bland-Altman Analyses					
		Mean Difference			Regression		
		FFQ - Recall	SD	$P_{difference}$	Intercept	Slope	P_{slope}
Fermented beverages, g/day	17.1	-4.1	250.0	0.644	-12.9	0.01	0.558
Coffee, g/day	19.5	9.3	220.8	0.231	-17.7	0.1	0.035
Beer, g/day	-73.9	-2.9	120.4	0.501	3.7	-0.1	0.004
Wine, g/day	0.3	-10.3	68.1	<0.0001	-0.5	-0.1	<0.0001
Fermented cereals/grains, g/day	0.02	-13.9	45.0	<0.0001	-11.9	-0.01	0.668
Brown bread, g/day	50.9	-0.6	38.3	0.633	-3.6	0.1	0.010
White bread, g/day	-151.2	-9.9	27.4	<0.0001	1.6	-0.8	<0.0001
Wholegrain bread, g/day	-28.0	3.0	50.5	0.090	8.8	-0.1	0.053
Rye bread, g/day	196.8	0.2	11.4	0.693	1.1	-0.3	<0.0001
Other bread, g/day	556.0	0.8	19.8	0.229	2.0	-0.1	0.029
Pastries, g/day	-20.0	-0.4	6.1	0.098	0.8	-0.6	<0.0001
Chocolate, g/day	67.8	-3.4	10.6	<0.0001	-0.2	-0.4	<0.0001
Fermented dairy, g/day	55.0	-4.9	104.0	0.182	2.4	-0.04	0.182
Cheese, g/day	134.8	-0.1	24.7	0.921	-9.3	0.3	<0.0001
Yoghurts, g/day	186.6	11.9	89.7	<0.0001	11.7	0.00	0.941
Quark, g/day	-33.1	-10.9	33.9	<0.0001	-2.2	-1.0	<0.0001
Buttermilk, g/day	15756.6	41.9	74.6	<0.0001	0.9	1.9	<0.0001
Non-fermented dairy, g/day	106.6	15.9	113.3	<0.0001	11.1	0.03	0.311
Butter, g/day	-18.7	0.02	6.1	0.921	-0.9	0.3	<0.0001
Cream, g/day	72.6	-4.9	12.9	<0.0001	-0.2	-0.9	<0.0001
Ice cream, g/day	-7.0	-1.7	14.7	0.001	3.2	-0.7	<0.0001
Milk, g/day	65.3	25.1	112.8	<0.0001	20.2	0.04	0.246
Non-fermented soya, g/day	209.7	-1.8	34.6	0.143	1.9	-0.4	<0.0001

FFQ, food frequency questionnaire; SD, standard deviation.

[a] Bland-Altman analyses were performed using the mean energy-adjusted intake values for each food group.

[b] Percent difference is calculated using [(FFQ - Recall)/Recall] x 100% for each individual and subsequently averaged for the entire group.

Table S3. Attenuation factors for the reference 24-hour recall compared to the food frequency questionnaire

	Attenuation Factors (λx)[a]													
	Crude							Energy-Adjusted						
	Number of 24-h Recalls							Number of 24-h Recalls						
Foods and Food Groups	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Fermented beverages	0.83	0.88	0.91	0.93	0.94	0.95	0.96	0.81	0.87	0.9	0.92	0.93	0.94	0.95
Coffee	0.86	0.91	0.93	0.95	0.96	0.96	0.97	0.85	0.90	0.92	0.94	0.95	0.96	0.96
Beer	0.71	0.82	0.89	0.93	0.96	0.99	1.00	0.60	0.69	0.74	0.77	0.80	0.82	0.83
Wine	0.65	0.75	0.82	0.86	0.89	0.92	0.94	0.64	0.74	0.80	0.84	0.87	0.89	0.90
Fermented cereals/grains	0.58	0.67	0.73	0.76	0.79	0.81	0.83	0.51	0.6	0.66	0.71	0.74	0.77	0.79
Brown bread	0.32	0.43	0.52	0.6	0.67	0.72	0.77	0.28	0.37	0.44	0.50	0.54	0.58	0.62
White bread	0.27	0.35	0.42	0.47	0.52	0.56	0.59	0.26	0.33	0.39	0.44	0.48	0.51	0.53
Wholegrain bread	0.47	0.59	0.67	0.73	0.78	0.82	0.85	0.47	0.58	0.66	0.71	0.76	0.79	0.82
Rye bread	0.59	0.71	0.8	0.86	0.91	0.94	0.97	0.54	0.63	0.69	0.74	0.77	0.79	0.81
Other bread	0.19	0.27	0.35	0.41	0.47	0.53	0.57	0.20	0.28	0.35	0.41	0.46	0.51	0.56
Pastries	0.23	0.33	0.42	0.5	0.58	0.65	0.72	0.25	0.34	0.43	0.51	0.57	0.63	0.69
Chocolate	0.32	0.41	0.48	0.54	0.59	0.62	0.65	0.33	0.41	0.48	0.53	0.56	0.60	0.62
Fermented dairy	0.52	0.59	0.64	0.67	0.70	0.71	0.73	0.60	0.70	0.76	0.8	0.83	0.86	0.88
Cheeses	0.42	0.53	0.61	0.67	0.71	0.75	0.78	0.41	0.52	0.59	0.65	0.7	0.73	0.76
Yoghurts	0.53	0.67	0.76	0.83	0.88	0.93	0.96	0.53	0.66	0.75	0.82	0.87	0.92	0.95
Quark	0.15	0.18	0.21	0.22	0.23	0.24	0.25	0.14	0.17	0.20	0.21	0.22	0.23	0.24
Buttermilk[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Non-fermented dairy	0.69	0.79	0.86	0.9	0.93	0.96	0.98	0.69	0.79	0.85	0.89	0.92	0.94	0.96
Butter	0.65	0.77	0.84	0.89	0.92	0.95	0.97	0.64	0.74	0.81	0.86	0.89	0.91	0.94
Cream	0.33	0.39	0.43	0.45	0.47	0.49	0.50	0.32	0.38	0.42	0.45	0.47	0.48	0.49
Ice cream	0.20	0.29	0.37	0.44	0.51	0.57	0.63	0.18	0.26	0.33	0.39	0.45	0.50	0.55
Milk	0.57	0.63	0.66	0.69	0.7	0.71	0.72	0.57	0.63	0.66	0.69	0.70	0.71	0.72
Non-fermented soya	0.6	0.66	0.7	0.72	0.74	0.75	0.76	0.61	0.67	0.70	0.72	0.74	0.75	0.76

[a] Crude and energy-adjusted attenuation factors reported for each number of 24-h recalls, ranging from 2 to 8.

[b] For buttermilk, the error model did not converge due to the low variance of the person-specific biases compared to the within- and between-person variances and are therefore not reported.

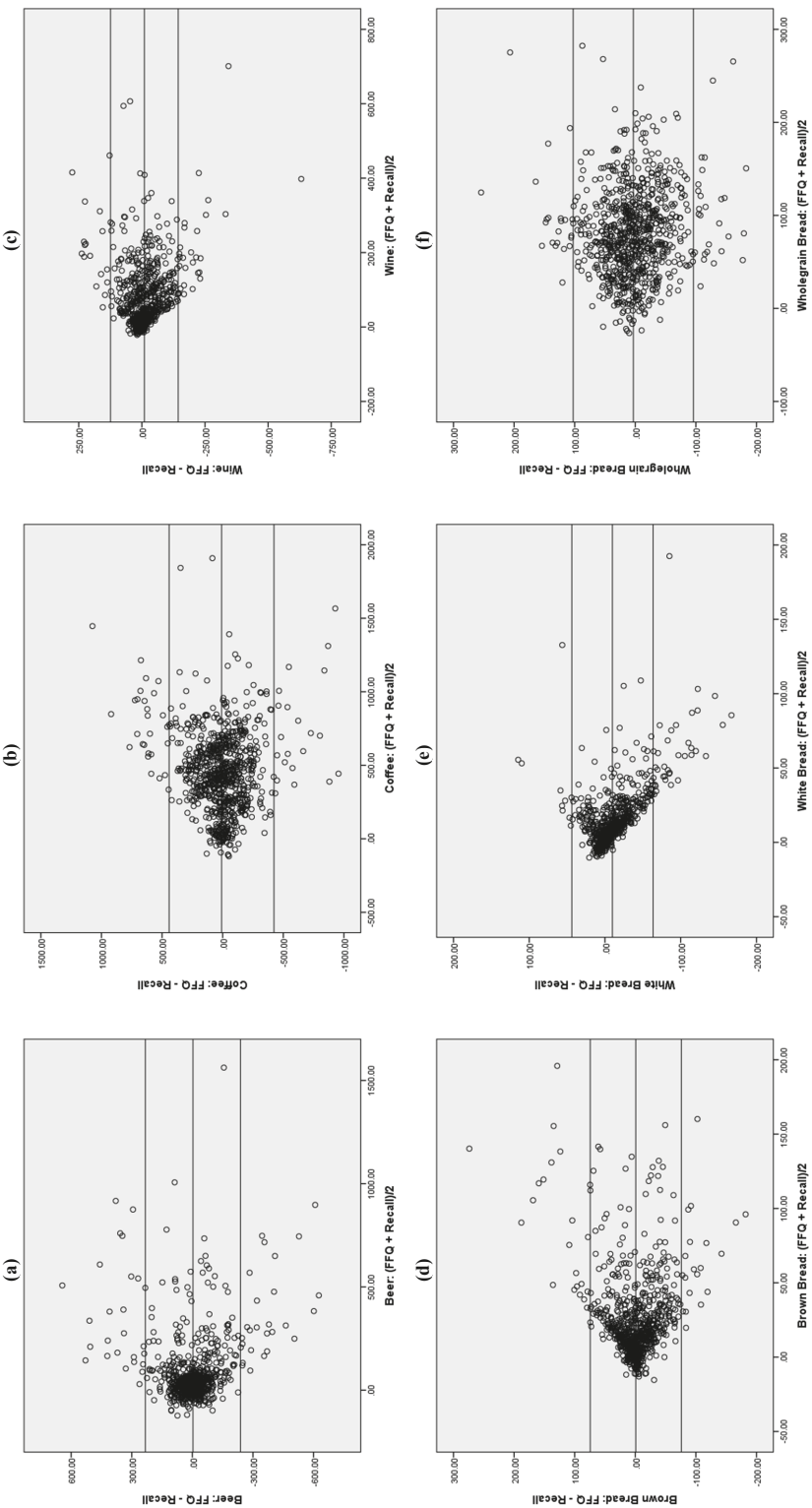
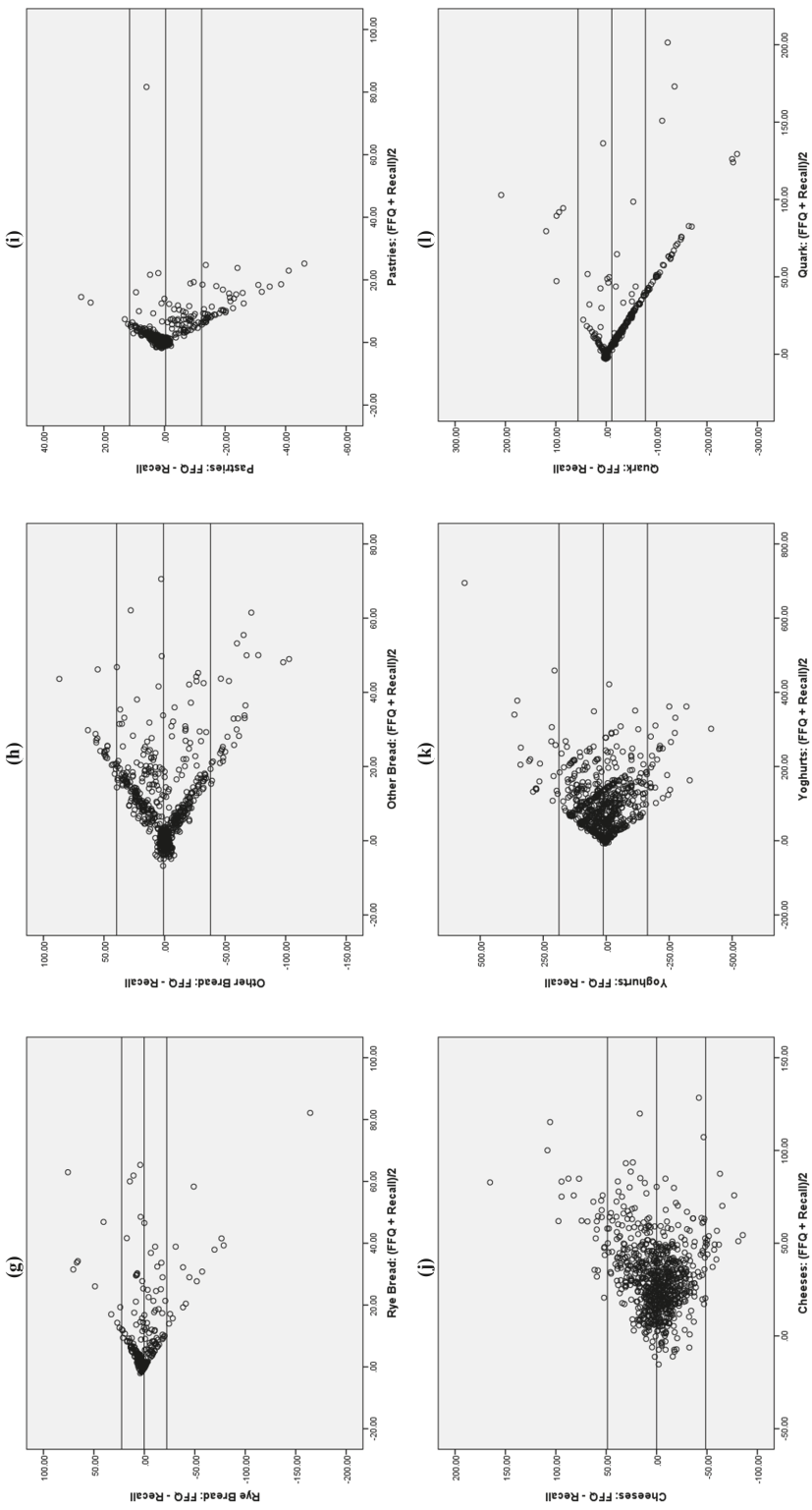
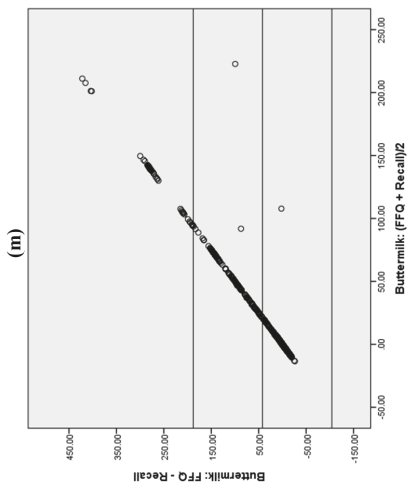


Figure S1. Bland-Altman plots demonstrating relative validity of the FFQ versus 24-h recalls for fermented food subgroups. Group-level relative validity shown for the following subgroups: (a) beer, (b) coffee, (c) wine, (d) brown bread, (e) white bread, (f) wholegrain and wholemeal bread, (g) rye bread, (h) other bread, (i) pastries, (j) cheeses, (k) yoghurts, (l) quark, and (m) buttermilk. The middle line indicates the mean difference, while the upper and lower lines indicate the 95% confidence intervals, respectively [calculated as: mean ± (standard deviation of the mean difference X 1.96)].


(g)
Rye Bread: FFQ - Recall
Rye Bread: (FFQ + Recall)/2
(h)
Other Bread: FFQ - Recall
Other Bread: (FFQ + Recall)/2
(i)
Pastries: FFQ - Recall
Pastries: (FFQ + Recall)/2
(j)
Cheeses: FFQ - Recall
Cheeses: (FFQ + Recall)/2
(k)
Yoghurts: FFQ - Recall
Yoghurts: (FFQ + Recall)/2
(l)
Quark: FFQ - Recall
Quark: (FFQ + Recall)/2


(m)
Buttermilk: FFQ - Recall
450.00
350.00
250.00
150.00
50.00
-50.00
-150.00
-50.00
.00
50.00
100.00
150.00
200.00
250.00
Buttermilk: (FFQ + Recall)/2

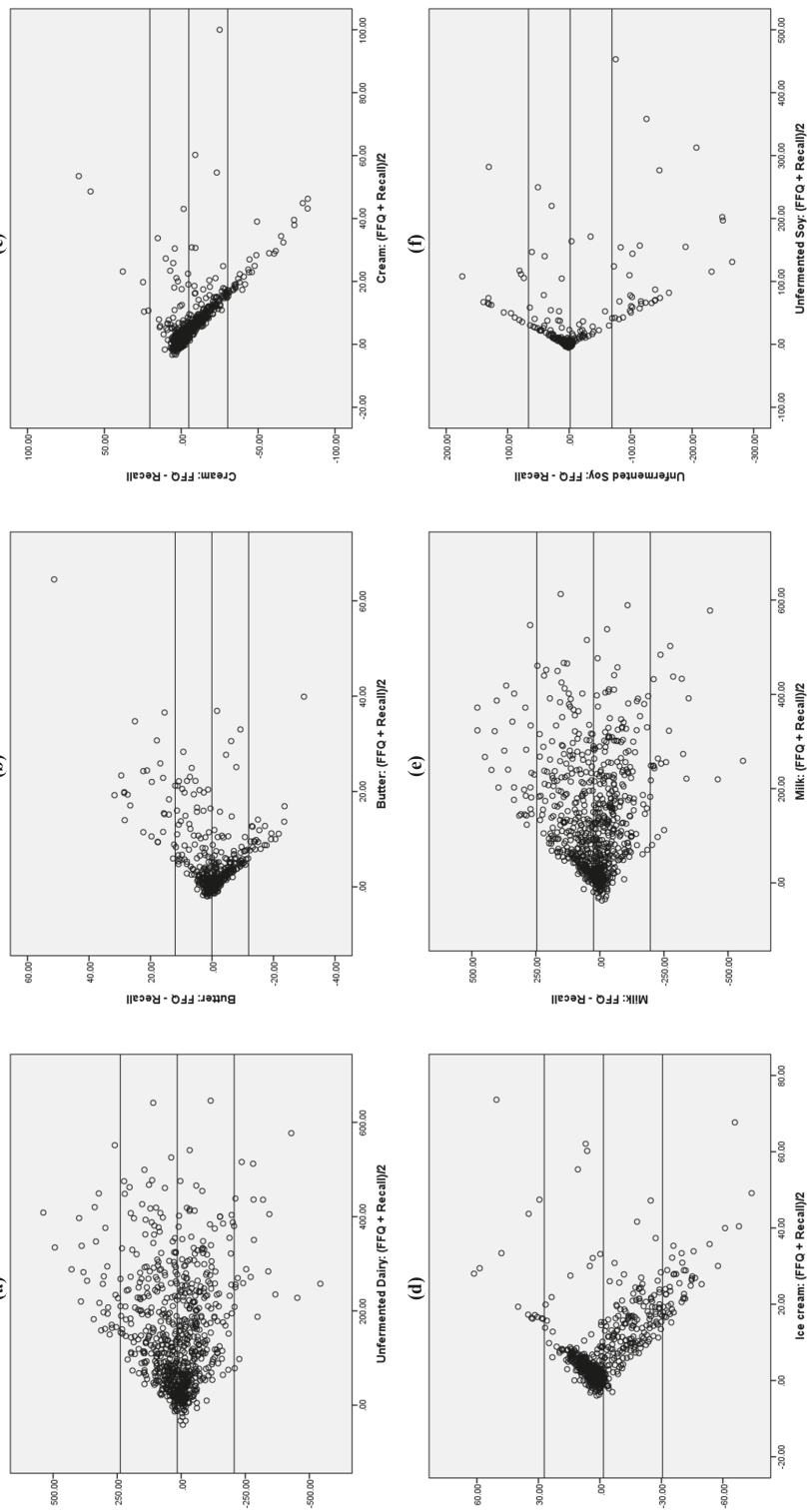


Figure S2. Bland-Altman plots demonstrating relative validity of the FFQ versus 24-h recalls for non-fermented foods. Group-level relative validity shown for: (a) non-fermented dairy, (b) butter, (c) cream, (d) ice cream, (e) milk, (f) non-fermented soya. The middle line indicates the mean difference, while the upper and lower lines indicate the 95% confidence intervals, respectively [calculated as: mean ± (standard deviation of the mean difference X 1.96)].

Table S4. Sex-specific Spearman's correlations for fermented and non-fermented foods

	Spearman's Rank Correlation Coefficient (r_s)			
	Male (n = 425)		Female (n = 384)	
Foods and Food Groups	**Crude**	**Energy-Adjusted**	**Crude**	**Energy-Adjusted**
Fermented beverages	0.72**	0.73**	0.80**	0.80**
Coffee	0.69**	0.69**	0.79**	0.79**
Beer	0.67**	0.66**	0.49**	0.57**
Wine	0.75**	0.69**	0.74**	0.73**
Fermented cereals/grains	0.65**	0.64**	0.64**	0.63**
Brown bread	0.27**	0.26**	0.22**	0.23**
White bread	0.26**	0.28**	0.43**	0.42**
Wholegrain bread	0.56**	0.53**	0.66**	0.64**
Rye bread	0.47**	-0.16**	0.38**	0.63**
Other bread	0.09	0.27**	0.15**	0.22**
Pastries	0.24**	0.40**	0.17**	0.24**
Chocolate	0.38**	0.38**	0.32**	0.30**
Fermented dairy	0.70**	0.70**	0.65**	0.65**
Cheeses	0.45**	0.45**	0.46**	0.43**
Yoghurts	0.58**	0.59**	0.52**	0.52**
Quark	0.14**	-0.48**	0.14**	0.39**
Buttermilk	0.12*	0.37**	0.07	-0.23**
Non-fermented dairy	0.66**	0.65**	0.70**	0.69**
Butter	0.45**	0.60**	0.52**	0.26**
Cream	0.21**	0.25**	0.18**	0.17**
Ice cream	0.22**	0.21**	0.26**	0.28**
Milk	0.64**	0.64**	0.70**	0.70**
Non-fermented soya	0.30**	-0.50**	0.46**	0.68**

FFQ, food frequency questionnaire. *, $p<0.05$; **, $p<0.01$.

CHAPTER 4

Evaluating the robustness of biomarkers of dairy food intake in a free-living population using single- and multi-marker approaches

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Abstract

Studies examining associations between self-reported dairy intake and health are inconclusive, but biomarkers hold promise for elucidating such relationships by offering objective measures of dietary intake. Previous human intervention studies identified several biomarkers for dairy foods in blood and urine using non-targeted metabolomics. We evaluated the robustness of these biomarkers in a free-living cohort in The Netherlands using both single- and multi-marker approaches. Plasma and urine from 246 participants (54±13 years) who completed a food frequency questionnaire were analyzed using liquid and gas chromatography-mass spectrometry. The targeted metabolite panel included 37 previously-identified candidate biomarkers of milk, cheese, and/or yogurt consumption. Associations between biomarkers and energy-adjusted dairy food intakes were assessed by a 'single-marker' generalized linear model, and stepwise regression was used to select the best 'multi-marker' panel. Multi-marker models that also accounted for common covariates better captured the subtle differences for milk (urinary galactose, galactitol; sex, body mass index, age) and cheese (plasma pentadecanoic acid, isoleucine, glutamic acid) over single-marker models. No significant associations were observed for yoghurt. Further examination of other facets of validity of these biomarkers may improve estimates of dairy food intake in conjunction with self-reported methods, and help reach a clearer consensus on their health impacts.

Introduction

Dairy products are widely acknowledged as an essential component of a healthy, diverse diet. Over 6 billion people consume milk and dairy products globally (1), and rely on these foods as a source of critical nutrients for growth, development, and disease prevention. However, studies linking dairy and dairy fat intake with cardiovascular disease and cardiometabolic conditions have yielded inconsistent findings, and there is still no consensus among different systematic reviews and meta-analyses on this matter (2-4). Furthermore, several recent studies have indicated that fermented dairy products may be responsible for the cardioprotective effects of dairy foods (*e.g.*, (5, 6)). Fermentation of milk releases bioactive compounds, including some with anti-hypertensive and immunomodulatory properties, which can convey additional nutritive value (7). Certain fermented dairy products, such as yogurt, also contain live bacterial cultures that can modify the composition of the gut microbiota, thereby influencing the risk of developing obesity, type II diabetes, and general cardiovascular diseases (6, 8-10).

For epidemiologists, a challenging but necessary task lies in capturing the 'true' intake of dairy products, such that its relationship with disease risk can be accurately portrayed. A major limitation of current dietary assessment tools (*i.e.*, food frequency questionnaires (FFQ), 24-h recalls) is their reliance on subjective reporting by participants while food intake biomarkers (FIBs) offer an objective alternative, which can be used in conjunction with self-report tools to improve the dietary intake assessment of dairy food intake (11). The odd-chain fatty acids pentadecanoic acid (C15) and heptadecanoic acid (C17) have been used as markers for total dairy intake (in particular, dairy fat), and have been effectively used for adjusting intakes when examining role of dairy consumption on cardiometabolic diseases (12). However, these FIBs may not be as useful in capturing low-fat dairy products or distinguishing between specific dairy foods, and have also been criticized for being non-specific when assessing dairy intake in populations with high fish consumption (13). In addition, given the limitations of using single biomarkers to assess dietary intake of a food (*i.e.*, non-specific and high inter-individual variation), a multi-marker approach implying a combination of FIBs may improve the precision of the assessment (14). Recently, multi-marker models have been developed for various foods including wine (15) and cocoa (16). The sum of C15, C17, and/or *trans*-palmitoleic acid (t16:1n-7) have been previously used as biomarkers of dairy fat (17), but combined biomarkers reflecting the intake of specific dairy foods have not been exploited.

Eight criteria have been proposed for the validation of FIBs, one of which includes an evaluation of their robustness in both controlled intervention settings as well as free-living populations with complex, uncontrolled diets (18). A number of previous acute and short-term, controlled human intervention studies have already been conducted in our laboratory and resulted in the identification of several FIBs for milk, cheese, and yogurt, in serum and urine using untargeted metabolomics (19-22). These FIBs were identified using a combination of liquid chromatography mass spectrometry (LC-MS), gas chromatography mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR), since each platform offers unique advantages for the detection of specific compounds based on factors such as compound size, polarity, abundance, and ionization, and their combined use permits complementary coverage of the metabolome (23). In these studies, we also observed high inter-individual variability in the response of several candidate FIBs based on genetic variation. Specifically, postprandial responses for the lactose metabolites galactose, galactitol and galactonate in the serum and urine of healthy men following acidified milk intake were concordant with genetic lactase persistence (24). In another study, we found that the oligosaccharides Lewis A trisaccharide and Blood Group H disaccharide reflected milk intake, and hypothesized that this was dependent on the expression of galactoside 2-alpha-L-fucosyltransferase 2 (FUT2) or galactoside 2-alpha-L-fucosyltransferase 3 (FUT3) enzymes, which act in competition to influence the production of these metabolites (20).

In the current paper, we aimed to evaluate the robustness of previously-identified candidate FIBs for milk, cheese, and yogurt in a free-living population in the Netherlands, using both single- and multi-marker approaches, with investigation of known covariates and genetic targets. For comparison, we also evaluated the performance of C15 and/or C17 for predicting total dairy intake (as well as dairy intakes grouped by fermentation status and high/low fat content) in our population.

Materials and Methods

Study population

The Nutrition Questionnaires plus (NQplus) study is a prospective cohort study that was conducted in Dutch adults (primarily Caucasian, 20 to 70 years), living in or around Wageningen (The Netherlands). NQplus was initiated as an 'add-on' study to the National Dietary Assessment Reference Database (NDARD) project, to gather extensive data on participant demographics, lifestyle, medical history, and cardiometabolic health outcomes. A complete description of NQplus and NDARD has been provided elsewhere (25, 26). Briefly, 2048 men and women were recruited and included in the study between June 2011 and February 2013. Baseline measurements included an assessment of habitual dietary intake by FFQ and/or 24-h recall. Background demographics, health, anthropometric, and lifestyle data, along with fasting blood samples (total collected: EDTA plasma (6×0.5 mL+1×1.5 mL), citrate plasma (5×0.5 mL), serum (3×0.5 mL+2×1 mL) and one buffy coat sample) and 24-h urine samples (mean ± SD weight: 2282 ± 814 g), were also collected. All biosamples were stored in the biobank at -80 °C for future analysis. All measurements were performed according to a standardized protocol by trained research personnel. The study was approved by the ethical committee of Wageningen University and Research (protocol number NL34775.081.10) and conducted in agreement with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the start of the study.

Metabolomics analyses were performed on a sub-cohort of NQplus participants (n = 531), including participants with 'complete' dietary data (completion of one FFQ and at least two 24-h recalls) as well as a biosample collected within 14 days of completing either a FFQ or a 24-h recall. For the present study, we report on n = 246 participants who had a biosample collected within ±14 days of completing a FFQ (228 plasma and 216 urine samples). This criterion ensured that biosample collection occurred within the FFQ reference period of one month, providing an assessment of typical dietary intake that is not as sensitive to fluctuations in daily intake as repeated 24-h recall assessments.

Food frequency questionnaire and levels of dairy food consumption

A full description of the FFQ used to assess habitual dietary intake has been described in the study design papers for NQplus and NDARD (25, 26). The FFQ was self-administered and completed online using the open-source survey tool LimeSurvey (LimeSurvey Project Team/Carsten Schmitz, Hamburg, Germany), with ten frequency categories ranging from 'never' to '6–7 days per week'. Portion sizes were estimated using commonly used household measures. Total food intake (in g/d) was determined by multiplying consumption frequency by portion size as defined in the Dutch food composition tables (27). The majority of FFQ assessments were completed in the spring (n = 129) and summer (n = 94) months, with fewer assessments performed in the autumn (n = 9) or winter (n = 14) months. Although the intake levels of some dairy foods could be dependent on season, we did not observe a consistent trend for such differences. Out of 216 total food items in the FFQ, 39 were identified as dairy products, which were further classified into milk, cheese, yogurt, cream, butter, buttermilk, quark, and ice cream subgroups (**Table S1**). This FFQ has been previously validated for energy, fat, and various nutrients and food groups (28-30), including milk, yogurt, cheese, total fermented

dairy, and total non-fermented dairy (against multiple 24-h recalls) (31), which were used in the current study for evaluation of the respective candidate FIBs.

In addition, to evaluate the performance of C15, C17, and various biomarkers on dairy groups, a total dairy group was calculated from the combined intakes of all dairy products, a total fermented dairy group was calculated from the combined intakes of all fermented dairy products in the FFQ, and a total non-fermented dairy group was calculated from the combined intakes of all non-fermented dairy products in the FFQ. Ingredient lists of common grocery store items were consulted (where necessary) to ensure that specific dairy foods were truly fermented, and composite dishes containing a fermented dairy ingredient (*e.g.*, pizza with cheese) were excluded, as previously described (31). Total dairy, fermented dairy, and non-fermented dairy groups were further stratified into high-fat groups, which included all full-fat dairy products, and low-fat groups, which included semi-skim and skim dairy products (**Table S1**). Fat content (g/100g) for all dairy products was determined based on the values reported in the Dutch Food Composition Table (27) and classifications of products as skim, semi-skim, and full-fat were based on the guidelines set by the Dutch Dairy Commodities Act (see **Table S1**).

LC-MS sample preparation and analysis

Plasma and urine samples were analyzed using LC-MS and GC-MS. All samples were thawed on ice and kept at 4°C during analysis. Prior to LC-MS analysis, phospholipids were removed from plasma samples to limit ion suppression using the Phree filter (Phenomenex Inc., Torrance, CA). Urine samples were normalized based on the specific gravity as determined by the refractive index (refractometer RE40, Mettler Toledo, Switzerland), as described in Pimentel *et al.* (20). Briefly, urine samples were centrifuged at 1800 *g* for 10 minutes at 4°C. The supernatant was then diluted using milliQ water to a specific gravity of 1.0008 to ensure that sample measurement occurred within the linear dynamic range of the machine. LC-MS metabolomics analysis was performed using the UltiMate 3000 RS UPLC system (Thermo Fisher Scientific, Waltham, MA) with a Waters Acquity UPLC HSS T3 column (length 150 mm, diameter 2.1 mm, particle size 1.8 µm), coupled with the maXis 4G+ quadrupole time-of-flight mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). We ran a gradient from 5% to 95% of mobile phase A within 15 min at 0.4 ml/min. Mobile phase A consisted of Milli-Q water with 0.1% formic acid and mobile phase B consisted of acetonitrile with 0.1% formic acid. The column was heated to 35°C with a post column cooler set to 25°C. The resulting system pressure was ~600 bar, dependent on the actual composition of the mobile phase at the specific time. The mass spectrometer electrospray interface was operated in positive ion mode and spectra were recorded from 75 to 1500 *m/z*. Collision-induced dissociation was performed using energies from 20 to 70 eV. 5 uL of de-phosphoralized plasma or normalized urine from each sample were injected. All samples were injected once. Quality control (QC) pools were prepared from plasma or urine samples by mixing all samples of each sample type at equal volume. QC samples were injected at five sample intervals for signal drift correction. Blanks (consisting of ultrafiltered LC-MS-grade water) were also injected at the beginning and end of each batch for detection of contaminants. Progenesis QI (v.2.3.6198.24128, NonLinear Dynamics Ltd., Newcastle upon Tyne, United Kingdom) was used for retention time correction, peak-picking, deconvolution, adducts annotation, and normalization (default automatic sensitivity and without minimum peak width). The intensity and the detection limit of the candidate FIBs was also performed by Progenesis QI with the setting "default". The software does not limit the detection at a certain intensity, but respects the noise level and presence of an isotopic pattern.

GC-MS sample preparation and analysis

Plasma and urine samples were prepared for GC-MS analysis as previously described (19, 22). Urine samples were normalized prior to analysis using the refractive index methods described above for the LC-MS analysis. For each 100 µL plasma sample, 50 µL of an internal standard solution (labelled D-fructose, U-13C6,

99%, Cambridge Isotope Laboratories, Inc., Cambridge, UK, c ≈ 0.16 mg/mL in water) was added, followed by precipitation with 300 µL cold methanol, centrifugation, transfer of supernatant (370 µL), and drying using a vacuum centrifuge. For each 100 µL urine sample, 50 µL of an internal standard solution (labelled D-fructose) was added and dried using a vacuum centrifuge. The samples further underwent a two-step derivatization (methoximation with O-Methylhydroxylamine hydrochloride followed by silylation with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA)) and subjected to analysis on a GC-MS 7890B/MS5977A (Agilent Technologies, Santa Clara, CA, U.S.) with a CombiPAL autosampler (CTC-Analytics AG, Zwingen, Switzerland) and a DB-5 ms fused silica capillary column (60 m, 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies, Basel, Switzerland). The samples were injected using a multimode injector according to the following temperature program: initially 90 °C, heating rate 900 °C/min until 280 °C, hold for 5 min and cooled at rate of -30 °C/min, and kept at 250 °C. The oven program was as follows: initial temperature 70 °C for 2 min, increase up to 160 °C at a rate of 5 °C/min, increase to 300 °C at a rate of 10 °C/min, which was held for 36 min, equilibration time 1 min. MS detection mass ranged from 28.5 to 600 Da, MS source temperature was 230 °C, and MS Quad temperature was 150 °C. Electron ionisation was performed with 70 eV. QC samples were prepared beforehand by mixing all plasma samples at equal volumes. Each batch was initiated by five injections of QC samples for equilibration and after every 5th plasma sample a fresh QC was injected. At start and end of each batch, a blank sample (milliQ water) was included. QC samples and blank samples underwent the same sample preparation as plasma samples.

Agilent data files acquired from GC-MS analysis were deconvoluted and converted into CEF files using Agilent MasshunterProfinder (Agilent Technologies, Santa Clara, U.S.). Data files were further processed in Agilent Mass Profiler Professional (Agilent Technologies, Santa Clara, U.S.) to perform, alignment and compound identification. In the resulting list containing the deconvoluted features, features with retention time before 10 min were removed (reagents region). All markers selected based on deconvoluted data were further evaluated using a targeted approach in order to optimize integration. Using RI, quantifier and qualifier ion retrieved from deconvoluted data, the suggested markers were analyzed in MassHunter Quantitative Analysis (Agilent Technologies, Santa Clara, U.S.). The peak integration was checked in each sample individually. Responses from the quantifier ion of marker compounds were normalized with the response of the quantifier ion of internal standard labelled d-Fructose Peak 1 (ion 279).

Previously-identified candidate biomarkers, analytical standards and reagents

Candidate FIBs for milk, cheese, and yoghurt were previously identified in serum and urine using non-targeted metabolomics, where the most discriminant FIBs were selected using Projections to Latent Structures Discriminant Analysis (PLS-DA) (details and figures reported elsewhere) (19-22). A list of these FIBs is provided in **Table S2**. Where possible, we aimed to evaluate these previously-identified candidate FIBs for milk, cheese, and yogurt in the biosample and using the same analytical platform by which they were originally identified. FIBs that were previously identified in serum were targeted in plasma. FIBs that were previously identified using NMR could not be assessed by the same platform in the present study; thus we used GC-MS as a substitution platform for the identification of most of these FIBs. All solvents and reagents for metabolomics analysis were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland).

For LC-MS, the Human Metabolome Database (32) and the National Institute of Standards and Technology database (NIST v14) were used to screen the identity of metabolites with a 10 ppm mass accuracy threshold. Compound identities were then confirmed with the injection of authentic standards with a RT window of 20%. A list of all standards suppliers is provided in **Table S3**. For GC-MS, an internal database was used for identification of targeted biomarkers. In the case that stereoisomeric forms of selected discriminating features were identified, the peak with higher response was further evaluated. Details of the

identification features of compounds analyzed from LC-MS and GC-MS are presented in **Tables S4** and **S5**, respectively.

Determination of lactase, FUT2, and FUT3 expression

Since the digestion of lactose and levels of lactose metabolites (galactose, galactonate, galactitol, galactono-1,5-lactone) are dependent on the presence of a functional lactase enzyme in adults (24), we evaluated the prevalence of the lactase persistent genotype in our population, and its influence on the utility of lactose metabolites as FIBs of milk intake. Similarly, the status of galactoside 2-alpha-L-fucosyltransferase 2 (FUT2) and galactoside 2-alpha-L-fucosyltransferase 3 (FUT3) enzymes, which determines the secretion of blood group antigens Lewis A trisaccharide and Blood Group H disaccharide that were previously proposed as candidate FIBs for milk intake (20), were also evaluated. We utilized whole-genome sequencing data that was performed for 737 NQplus participants, of which $n = 110$ overlapped with our validation sub-cohort. DNA was extracted from the blood samples of these participants using a Puregene 5Prime kit (Qiagen, Germantown, MD) and sequenced using the Illumina OmniEspress chip (Illumina Inc., San Diego, CA). We screened the single nucleotide polymorphisms (SNPs) data that was obtained through sequencing against a comprehensive list of SNPs associated with lactase persistence in the literature (33-39), which encompassed the common known functional SNPs rs4988235, rs182549, and rs41380347, as well as a number of rare variants (accession numbers: rs41456145, rs145946881, rs41525747, rs869051967, ss820496565, rs4988233, rs527991977, rs4954492, rs56348046, rs4954490, rs759157971). In addition, known SNPs for the *FUT2* (rs601338, rs1047781, rs281377, rs200157007) and *FUT3* (rs28362459, rs3745635, rs3894326, rs812936, rs778986) genes were also screened. From the screening, rs4988235 (13910C>T) was identified among the SNPs of the *LCT* gene and rs182549 (22018G>A) was identified for the upstream *MCM6* gene, both influencing lactase status, while rs601338 (G428A) was found for *FUT2* and rs778986 (C314T) for *FUT3*. Phenotypes for lactase (persistent and non-persistent) and FUT2/FUT3 status (secretors, non-secretors, and Lewis negative) were determined based on the SNPs.

Statistical analysis

Participant characteristics are shown for the total population as well as stratified for sex as mean (SD), median (IQR) or n (%). Exploratory analyses were performed and metabolomics sample outliers, defined as observations clearly falling outside Hotelling's T^2 tolerance eclipse (95% confidence interval) in the principal component analysis (PCA) score plot, were identified and excluded ($n = 23$ LC-MS plasma, $n = 2$ LC-MS urine, and $n = 24$ GC-MS plasma).

Differences in levels of FIBs by quintiles of intake for dairy groups and dairy foods (for the total population, and sex-specific) were assessed by a Kruskal-Wallis test followed by a *post-hoc* Conover-Iman pairwise comparison test ($p \leq 0.05$ as significance threshold). Spearman's correlation coefficients (r_s) were generated to analyse metabolite levels by continuous energy-adjusted g/d intakes (for the total population and by sex). Correlation coefficients of ≥0.50 were considered to be good, 0.20 to 0.49 as acceptable, and <0.20 as poor (40). In addition, generalized linear models (GLM) with quasi-Poisson distribution were used to evaluate the performance of the candidate biomarkers in a 'single-marker' model in predicting the intake of different dairy foods. To avoid the use of negative values in the GLM, energy-adjusted intakes of dairy foods (g/d) were first offset by adding the minimum absolute intake value to all intake values of a food. Since the metabolite concentrations were compositional (*i.e.*, they are expressed as relative abundance), we normalized metabolite concentrations prior to analysis using a centered log ratio (CLR) transformation (41-43). CLR transformation was performed for the metabolite data using compositions R package (v2.0-0) (44).

To evaluate whether a 'multi-marker' panel consisting of a combination of FIBs performed better than single FIBs in predicting intakes, stepwise regression models (forwards and backwards) were generated for biomarkers per platform and per biosample for milk, cheese, and yogurt. For dairy groups (total dairy,

fermented dairy, non-fermented dairy, and their high- and low-fat variations), a combination of plasma C15 and C17 was investigated. Further, FIBs with significant spearman's correlations $r_s > 0$ for intake of fermented dairy products (total, high-fat, and low-fat fermented dairy, cheese, yoghurt) and $r_s < 0$ for intake of non-fermented dairy products (total, high-fat, and low-fat non-fermented dairy, milk) were further modelled using stepwise regression and a multi-marker approach to investigate which FIBs can help distinguish between fermented and non-fermented dairy intake. The best multi-marker models were selected based on the lowest quasi-Akaike Information Criterion (qAIC) value determined using the R package MuMIn (v1.43.17) (45) and presented in this paper. Multicollinearity of biomarkers were evaluated using the variance inflation factor (VIF), where VIF>5 indicates potentially severe correlation between predictor variables, as confirmed/verified by pairwise correlations between biomarkers. In multi-marker models where high multicollinearity between several variables were observed, colinear variable(s) with the highest VIF were removed. Several categorical covariates were also added to adjust the regression models (0, 1): sex (male, female), BMI (normal weight <25 kg/m^2, overweight/obese ≥25/m^2), and age (<55 years, ≥55 years (median split)).

For cross-validation of both the single-marker and multi-marker models, the dataset was randomly split into training (80%) and test datasets (20%). Spearman's correlations between the actual and predicted values (r_{ap}) were calculated to assess the strength and direction of the associations between these data, and performance accuracy of the models was further assessed by a coefficient of determination (R^2) and mean absolute error (MAE), which was determined using the R package MLmetrics (v1.1.1) (46). All statistics were performed in R (Version 3.6.3) (47). For all models, the level of significance was set at $p \leq 0.05$.

Results

Characteristics of the validation sub-cohort

The characteristics of the validation sub-cohort is provided in **Table 1**. The majority of the participants were men (67%). The mean age of the participants was 54 ± 13 years, with men (56 years) being significantly older than women (51 years). A majority of men (63%) and almost half (46%) of women had a body mass index (BMI) corresponding to overweight or obese (≥25 kg/m^2), which were also significantly different between sexes. A higher number and proportion of women (*n* = 12, 15%) than men followed a diet within the month preceding the study. The vast majority (95%) of participants were categorized as lactase persistent. Further, based on FUT2/FUT3 enzyme functional status, a majority of participants (with similar proportions in men and women) were classified as 'secretors' (79%), while a smaller percentage were 'non-secretors' (17%) and Lewis negative (4%).

Table 1. General characteristics of the participants[a]

	All (*n* = 246)	**Men (*n* = 165)**	**Women (*n* = 81)**	***p*-value**
Age, years	54.4 ± 12.5	55.9 ± 11.6	51.2 ± 13.6	0.01**
BMI, kg/m^2	25.9 ± 3.9	26.1 ± 3.6	25.4 ± 4.4	0.18
BMI-category, *n* (%)				0.010**
<25 kg/m^2	105 (42.7)	61 (37.0)	44 (54.3)	
≥25 kg/m^2	141 (57.3)	104 (63.0)	37 (45.7)	
Waist circumference, cm	92.5 ± 11.6	95.8 ± 10.5	85.6 ± 10.7	<0.001***
Education, *n* (%)				0.38
Low	19 (7.7)	12 (7.3)	7 (8.8)	
Intermediate	77 (31.3)	49 (29.7)	28 (35.0)	
High	149 (60.6)	104 (63.0)	45 (56.2)	
Smoking status, *n* (%)				0.09
Never	119 (48.4)	71 (46.4)	48 (63.2)	
Former	85 (34.6)	65 (42.5)	20 (26.3)	
Current	25 (10.2)	17 (1.1)	8 (10.5)	

Disease history, *n* (%)				
Cancer	11 (4.5)	5 (3.0)	6 (7.4)	0.12
Diabetes	6 (2.4)	5 (3.0)	1 (1.2)	0.39
Heart attack	7 (2.8)	6 (3.6)	1 (1.2)	0.29
Hypertension	60 (24.4)	44 (26.7)	16 (19.8)	0.47
High cholesterol	52 (21.1)	38 (23.0)	14 (17.3)	0.58
Stroke	2 (0.8)	1 (0.6)	1 (1.2)	0.61
Diet during past month, *n* (%)				<0.001***
No	228 (92.7)	159 (96.4)	69 (85.2)	
Yes, always	9 (3.7)	1 (0.6)	8 (9.9)	
Yes, sometimes	9 (3.7)	5 (3.0)	4 (4.9)	
Lactase status, *n* (%)				1.00
Persistent	104 (94.5)	81 (94.2)	23 (95.8)	
Non-persistent	6 (5.5)	5 (5.8)	1 (4.2)	
FUT2/FUT3 status, *n* (%)				0.41
Secretor (Le a^-b^+)	87 (79.1)	69 (80.2)	18 (75.0)	
Non-secretor (Le a^+b^-)	19 (17.3)	13 (15.1)	6 (25.0)	
Lewis negative (Le a^-b^-)	4 (3.6)	4 (4.7)	0 (0)	

BMI, body mass index; FUT2, galactoside 2-alpha-L-fucosyltransferase 2; FUT3, galactoside 2-alpha-L-fucosyltransferase 3; SD, standard deviation. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

[a] Values are presented as mean ± SD, unless otherwise specified. Missing values: lactase, FUT2, and FUT3 status (n = 136), education (n = 1), smoking status (n = 17). Differences in characteristics between sexes were assessed using the t-test (for continuous variables), or chi-squared test (for categorical variables).

Intake levels of different dairy products

Quintiles of median energy-adjusted intakes for different dairy groups and individual dairy foods are presented in **Table 2**. All participants consumed at least one type of dairy product, but some participants did not consume low-fat non-fermented dairy (n = 65), high-fat fermented dairy (n = 41), milk (n = 37), yogurt (n = 34), high-fat non-fermented dairy (n = 9), cheese (n = 4), high-fat dairy (n = 3), total non-fermented dairy (n = 2), low-fat fermented dairy (n = 2), low-fat dairy (n = 1), and/or any fermented dairy (n = 1). Median intakes in the highest quintile of consumption (Q5) ranged from 60 g/d for high-fat non-fermented dairy to 527 g/d for total dairy. For individual dairy foods, median intakes were highest for milk (303 g/d), followed by yogurt (193 g/d) and cheese (67 g/d). Sex-specific intake quintiles for the different dairy groups and individual dairy foods are presented in **Tables S6** and **Table S7** for men and women, respectively. Overall, men tended to have higher median intakes of high-fat dairy, total fermented dairy, high-fat fermented dairy, high-fat non-fermented dairy, low-fat fermented dairy, cheese, and yogurt compared to women (in the majority of quintiles). However, women had higher median intakes of low-fat dairy, total non-fermented dairy, low-fat non-fermented dairy, and milk in all quintiles, as well as total dairy (in all except lowest quintile) compared to men.

Table 2. Quintiles of intake for dairy groups and dairy foods (n = 246)

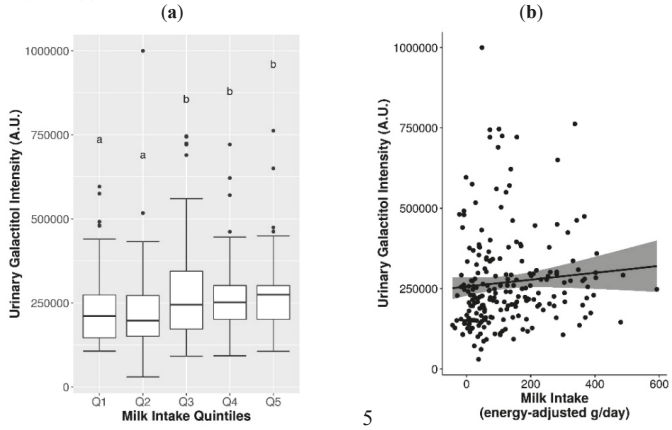
Food Group	**Median Energy-Adjusted Intakes in g/d**									
	n_c	**Q1 (n = 50)**	**n_c**	**Q2 (n = 49)**	**n_c**	**Q3 (n = 49)**	**n_c**	**Q4 (n = 49)**	**n_c**	**Q5 (n = 49)**
Total dairy	50	98 (71, 129)	49	214 (197, 235)	49	304 (279, 323)	49	372 (355, 394)	49	527 (469, 616)
High-fat dairy	47	10 (6, 15)	49	24 (21, 28)	49	42 (35, 48)	49	73 (64, 81)	49	135 (109, 163)
Low-fat dairy	49	43 (25, 59)	49	148 (119, 173)	49	242 (224, 257)	49	317 (304, 340)	49	480 (404, 590)
Total fermented dairy	49	41 (24, 49)	49	90 (69, 109)	49	143 (134, 161)	49	224 (204, 237)	49	334 (291, 393)
High-fat fermented dairy	9	3 (-1, 4)	49	9 (7, 10)	49	17 (14, 19)	49	37 (30, 45)	49	82 (65, 117)
Low-fat fermented dairy	48	15 (7, 23)	49	50 (40, 62)	49	108 (99, 124)	49	195 (158, 210)	49	304 (269, 370)
Total non-fermented dairy	48	12 (4, 22)	49	54 (44, 63)	49	103 (91 , 124)	49	179 (160 , 207)	49	322 (282 , 340)
High-fat non-fermented dairy	41	3 (1, 5)	49	10 (9 , 12)	49	18 (16 , 20)	49	31 (25 , 35)	49	60 (48 , 89)
Low-fat non-fermented dairy	0	-4 (-9, 5)	34	22 (14 , 32)	49	69 (55, 89)	49	146 (127, 173)	49	293 (263, 373)
Cheese	46	8 (4, 12)	49	19 (17, 21)	49	27 (24, 29)	49	43 (39, 47)	49	67 (58, 90)
Yogurt	16	0 (0, 5)	49	38 (22, 53)	49	83 (72, 96)	49	126 (105, 139)	49	193 (150, 212)
Milk	13	4 (-8, 14)	49	40 (29, 48)	49	87 (72, 108)	49	162 (144, 191)	49	303 (272, 371)

FFQ, food frequency questionnaire; n_c, number of consumers. Values are reported as median (IQR), unless otherwise specified.

Assessment of biomarkers for milk intake

Twenty-one candidate FIBs in **Table S2** that were previously found to be discriminant for milk intake were assessed, of which fifteen were detected in plasma and/or urine. When analyzed by quintiles of milk intake, a statistically significant increase in urinary galactitol was observed (Q3-5 vs. Q1-2, $p \leq 0.05$) (**Figure 1a**). Additional significant findings with an increasing trend were observed between FIBs and sex-specific quintiles of milk intake, including plasma phenylalanine and Lewis A trisaccharide in women, and urinary lactose and galactitol in men (**Figure S1**).

Figure 1. Significantly increased urinary galactitol levels by (a) quintiles of milk intake (significance between quintiles denoted by different letters, $p \leq 0.05$), and (b) continuous milk intake.



Spearman's correlations were weak and non-significant for the majority of milk FIBs, with the exception of urinary lactose ($r_s = 0.16$, $p \leq 0.05$) and galactitol ($r_s = 0.2$, $p \leq 0.05$) (**Table 3, Figure 1b**). Some sex-specific correlations were also observed between plasma phenylalanine, tyrosine, tryptophan, indole-3-acetic acid, and Lewis A trisaccharide with milk intake in women, and urinary lactose and galactitol with milk intake in men (**Table 3**). These associations were paralleled in several single-marker generalized linear regression (GLM) models, with significant results observed for urinary lactose, galactose, and galactitol in covariate-adjusted models (coefficients = 0.07-0.20, $r_{ap} = 0.17$-0.2, $R^2 = 0.03$-0.04, MAE ~ 93 g/d) (**Table 3**). Unadjusted and adjusted multi-marker models for milk derived from stepwise regression are presented in **Tables S8** and **S9**. Adjusted multi-marker models consisting of urinary galactose + galactitol + sex + BMI + age (analyzed by GC-MS) ($r_{ap} = 0.20$, $R^2 = 0.04$, mean absolute error (MAE) = 92 g/d), and plasma tryptophan + indole-3-propionic acid + sex (analyzed by LC-MS) ($r_{ap} = 0.25$, $R^2 = 0.06$, MAE = 102.8 g/d) had slightly improved performance accuracy for predicting milk intake compared to the single-marker models.

The role of lactase persistence status on the relative abundance of lactose and its metabolites in plasma and urine was further explored (**Figure S2**). As expected, plasma levels of lactose were low across all samples, due to the analysis of fasting samples, while urinary lactose was significantly higher in lactase non-persistent (LNP) individuals. While a higher relative abundance of all lactose metabolites were generally observed in lactase persistent (LP) individuals compared to LNP (with the possible exception of galactose in urine), the differences were not significant. Due to the low numbers of LNP individuals in our sub-cohort, further analyses of levels of lactose metabolites by quintiles of milk intake stratified by LP/LNP status was not possible.

Similarly, the role of FUT2 and FUT3 enzyme status on levels of Lewis A trisaccharide in plasma and Blood Group H disaccharide in plasma and urine was also explored (**Figure S3**). No significant differences were observed in plasma Lewis A trisaccharide between secretors and non-secretors. For Blood Group H disaccharide, while no significant between-group differences were detected in plasma (borderline $p = 0.057$), significantly higher levels were observed in urine for secretors compared to non-secretors ($p = 2.5 \times 10^{-9}$).

Table 3. Single-marker validation results for previously-identified candidate FIBs for milk

Biomarker	Analytical Platform (Biosample)[a]	Spearman's correlation coefficient (r_s)		Unadjusted GLM[b]						Adjusted GLM[b,c]					
				Coefficient	SE	*p*-value	r_{ap}	R^2	MAE	Coefficient	SE	*p*-value	r_{ap}	R^2	MAE
C15	GC-MS (P)	0.03	M: 0.05	(Int: 5.05)	(0.06)	(0.00***)	0.13	0.02	88.5	(Int: 5.24)	(0.12)	(0.00***)	0.09	0.01	89.0
			W: 0.00	0.04	0.12	0.76				0.08	0.12	0.49			
C17	GC-MS (P)	0.02	M: 0.06	(Int: 5.05)	(0.06)	(0.00***)	-0.12	0.01	88.5	(Int: 5.24)	(0.12)	(0.00***)	0.12	0.01	88.8
			W: 0.00	0.03	0.14	0.82				0.08	0.14	0.55			
Phenylalanine	LC-MS (P)	0.11	M: 0.03	(Int: 5.06)	(0.05)	(0.00***)	0.25	0.06	104.0	(Int: 5.29)	(0.11)	(0.00***)	0.47	0.22	99.8
			W: 0.32**	0.08	0.05	0.10				0.09	0.05	0.07			
Tyrosine	LC-MS (P)	0.08	M: 0.01	(Int: 5.06)	(0.05)	(0.00***)	0.15	0.02	104.5	(Int: 5.29)	(0.11)	(0.00***)	0.53	0.28	100.5
			W: 0.25*	0.08	0.05	0.12				0.09	0.05	0.06			
Tryptophan	LC-MS (P)	0.12	M: 0.06	(Int: 5.06)	(0.05)	(0.00***)	0.16	0.03	105.6	(Int: 5.28)	(0.11)	(0.00***)	0.38	0.14	101.7
			W: 0.25*	0.11	0.06	0.10				0.11	0.06	0.09			
Indole-3-propionic acid	LC-MS (P)	0.04	M: -0.03	(Int: 5.07)	(0.05)	(0.00***)	0.05	0.00	106.4	(Int: 5.27)	(0.11)	(0.00***)	0.40	0.16	102.7
			W: 0.16	0.02	0.06	0.68				0.02	0.06	0.75			
Indole-3-acetic acid	LC-MS (P)	0.10	M: -0.01	(Int: 5.06)	(0.05)	(0.00***)	-0.08	0.01	106.3	(Int: 5.26)	(0.11)	(0.00***)	0.25	0.07	103.7
			W: 0.29*	0.09	0.07	0.18				0.08	0.07	0.22			
Lactose	GC-MS (U)	**0.16***	**M: 0.23****	(Int: 5.12)	(0.05)	(0.00***)	0.16	0.03	91.8	**(Int: 5.30)**	**(0.11)**	**(0.00***)**	**0.20**	**0.04**	**92.7**
			W: 0.08	0.12	0.06	0.05				**0.13**	**0.06**	**0.03***			
	GC-MS (P)	-0.01	M: -0.05	(Int: 5.05)	(0.06)	(0.00***)	0.09	0.01	88.3	(Int: 5.23)	(0.12)	(0.00***)	0.10	0.01	88.8
			W: 0.11	0.06	0.09	0.55				0.05	0.09	0.59			
Galactose	GC-MS (U)	0.04	M: 0.11	(5.12)	(0.05)	(0.00***)	0.22	0.05	94.3	**(Int: 5.33)**	**(0.11)**	**(0.00***)**	**0.21**	**0.04**	**93.0**
			W: 0.10	0.04	0.03	0.20				**0.07**	**0.03**	**0.04**			
	GC-MS (P)	-0.02	M: -0.02	(5.05)	(0.06)	(0.00***)	-0.08	0.01	89.4	(Int: 5.24)	(0.12)	(0.00***)	0.08	0.01	88.5
			W: -0.02	-0.11	0.27	0.68				-0.12	0.27	0.65			
Galactitol	GC-MS (U)	**0.20****	**M: 0.23****	**(Int: 5.12)**	**(0.05)**	**(0.00***)**	**0.17**	**0.03**	**93.6**	**(Int: 5.28)**	**(0.11)**	**(0.00***)**	**0.17**	**0.03**	**93.3**
			W: 0.07	**0.21**	**0.10**	**0.03**				**0.20**	**0.10**	**0.04**			
	GC-MS (P)	0.00	M: -0.02	(Int: 5.05)	(0.06)	(0.00***)	-0.13	0.02	88.6	(Int: 5.24)	(0.12)	(0.00***)	0.14	0.02	88.7
			W: 0.05	0.01	0.12	0.94				0.06	0.12	0.60			
Galactonate	LC-MS (U)	0.14	M: 0.01	(Int: 5.12)	(0.05)	(0.00***)	0.12	0.01	96.7	(Int: 5.29)	(0.11)	(0.00***)	0.15	0.02	96.7
			W: 0.22	0.04	0.05	0.36				0.05	0.05	0.30			
	GC-MS (U)	0.04	M: 0.08	(Int: 5.12)	(0.05)	(0.00***)	0.19	0.04	95.6	(Int: 5.30)	(0.11)	(0.00***)	0.17	0.03	96.0
			W: 0.03	0.02	0.07	0.72				0.05	0.07	0.43			
	GC-MS (P)	0.02	M: 0.04	(Int: 5.05)	(0.06)	(0.00***)	0.13	0.02	87.8	(Int: 5.22)	(0.12)	(0.00***)	0.13	0.02	87.7

			W: 0.02	0.07	0.08	0.36				0.09	0.08	0.27			
Blood group H disaccharide	LC-MS (P)	-0.07	M: -0.10	(Int: 5.07)	(0.05)	(0.00***)	0.09	0.01	106.0	(Int: 5.27)	(0.11)	(0.00***)	0.38	0.15	102.3
			W: -0.06	-0.02	0.05	0.62				-0.02	0.05	0.63			
	LC-MS (U)	-0.05	M: 0.05	(Int: 5.12)	(0.05)	(0.00***)	0.04	0.00	97.1	(Int: 5.30)	(0.11)	(0.00***)	0.12	0.02	97.0
			W: -0.10	0.00	0.05	0.94				0.03	0.05	0.60			
Lewis A trisaccharide	LC-MS (P)	0.07	M: -0.01	(Int: 5.07)	(0.05)	(0.00***)	-0.01	0.00	106.4	(Int: 5.27)	(0.11)	(0.00***)	0.30	0.09	102.5
			W: 0.26*	0.00	0.04	0.97				0.00	0.04	0.93			
Hippurate	GC-MS (U)	-0.10	M: -0.02	(Int: 5.12)	(0.05)	(0.00***)	-0.15	0.02	95.7	(Int: 5.30)	(0.11)	(0.00***)	0.12	0.02	95.2
			W: -0.08	-0.04	0.11	0.73				0.01	0.11	0.91			
Methionine	GC-MS (P)	0.01	M: 0.03	(Int: 5.05)	(0.06)	(0.00***)	0.00	0.00	88.1	(Int: 5.25)	(0.12)	(0.00***)	0.10	0.01	87.5
			W: 0.05	0.08	0.13	0.53				0.13	0.13	0.32			

C15, pentadecanoic acid; C17, heptadecanoic acid; FIB, food intake biomarker; GC-MS, gas chromatography mass spectrometry; GLM, generalized linear model; LC-MS, liquid chromatography mass spectrometry; M, men; MAE, mean absolute error; P, plasma; r_{ap}, correlation between actual and predicted intake; SE, standard error; U, urine; W, women. Significant results are bolded: *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

[a] For the current study, biomarkers that were previously detected in serum were validated in plasma. For biomarkers that were previously detected using NMR, GC-MS was used as a substitution platform. A few biomarkers were not visible (asparagine (LC-MS plasma), taurine (LC-MS plasma), allantoin (GC-MS urine)) or not detected (galactono-1,5-lactone (GC-MS plasma and urine), galactonate (LC-MS plasma), gluconic acid and delta-Gluconolactone (LC-MS plasma)) and were therefore not included in the current validation.

[b] Intercept (Int) values for the models are provided in brackets.

[c] Adjusted for age, sex, and BMI.

Assessment of biomarkers for cheese intake

Sixteen previously-identified candidate FIBs for cheese intake are presented in **Table S2**, of which 14 were detected in plasma and/or urine samples of the current cohort (**Table 4**). For the total population, no significant differences in candidate FIB concentrations for cheese were observed across quintiles of cheese intake. However, when stratified into sex-specific intake quintiles, levels of urinary indole-3-lactic acid were significantly increased between quintiles of cheese intake in men, while plasma phenylalanyl-proline was increased between quintiles of cheese intake in women (**Figure S4**). For plasma proline in men, although also significant across quintiles, a decreasing trend was observed (**Figure S4**).

No significant positive Spearman's correlations were observed between FIBs and cheese intake on a continuous scale for any of the FIBs, but when stratified by sex, a significant correlation was revealed for urinary indole-3-lactic acid in men (**Table 4**). No significant single-marker models (adjusted or unadjusted) were generated for any of the FIBs for cheese intake. From the multi-marker models, a combination of plasma C15 + isoleucine + glutamic acid (analysed by gas chromatography-mass spectrometry (GC-MS)) yielded a significant model for predicting cheese intake, albeit with somewhat poor performance (r_{ap} = 0.16, R^2 = 0.03, MAE = 17 g/d) (**Table S8**). Inclusion of covariates in an adjusted multi-marker model did not further reveal combinations of biomarkers or biomarker and covariates that better predicts cheese intake (plasma C15 + isoleucine + glutamic acid was still the best model) (**Table S9**).

Table 4. Single-marker validation results for previously-identified candidate FIBs for cheese

Biomarker	Analytical Platform (Biosample)[a]	Spearman's correlation coefficient (r_s)		Unadjusted GLM[b]						Adjusted GLM[b,c]					
				Coefficient	SE	*p*-value	r_{ap}	R^2	MAE	Coefficient	SE	*p*-value	r_{ap}	R^2	MAE
C15	GC-MS (P)	0.12	M: 0.15	(Int: 3.91)	(0.03)	(0.00***)	0.21	0.04	15.5	(Int: 4.00)	(0.07)	(0.00***)	0.10	0.01	16.0
			W: 0.07	0.12	0.07	0.10				0.12	0.07	0.11			
C17	GC-MS (P)	0.08	M: 0.12	(Int: 3.91)	(0.03)	(0.00***)	0.19	0.04	15.8	(Int: 4.00)	(0.07)	(0.00***)	0.03	0.00	16.3
			W: 0.04	0.10	0.08	0.23				0.10	0.08	0.23			
3-Phenyllactic acid	GC-MS (U)	-0.11	M: 0.08	(Int: 3.97)	(0.03)	(0.00***)	-0.17	0.03	18.9	(Int: 4.099)	(0.07)	(0.00***)	0.16	0.02	18.5
			W: 0.08	0.01	0.04	0.78				0.00	0.04	0.98			
	GC-MS (P)	-0.05	M: 0.07	(Int: 3.91)	(0.03)	(0.00***)	-0.07	0.01	16.4	(Int: 3.99)	(0.07)	(0.00***)	-0.02	0.00	16.7
			W: -0.17	-0.06	0.08	0.45				-0.05	0.08	0.58			
3-Hydroxy-isobutyrate	GC-MS (P)	-0.04	M: -0.04	(Int: 3.91)	(0.03)	(0.00***)	-0.04	0.00	16.2	(Int: 3.99)	(0.07)	(0.00***)	0.03	0.00	16.6
			W: 0.01	0.00	0.07	0.99				0.00	0.08	0.95			
Phenylalanyl-proline	LC-MS (P)	0.05	M: 0.01	(Int: 3.88)	(0.04)	(0.00***)	-0.11	0.01	20.6	(Int: 4.06)	(0.08)	(0.00***)	0.07	0.01	20.3
			W: 0.18	0.04	0.03	0.17				0.05	0.03	0.09			
	LC-MS (U)	-0.07	M: -0.08	(Int: 3.97)	(0.03)	(0.00***)	-0.12	0.01	22.5	(Int: 4.11)	(0.07)	(0.00***)	-0.06	0.00	22.7
			W: 0.01	0.00	0.08	0.97				0.04	0.08	0.68			
Indole-3-lactic acid	LC-MS (P)	0.06	M: 0.02	(Int: 3.89)	(0.04)	(0.00***)	-0.07	0.01	20.8	(Int: 4.05)	(0.08)	(0.00***)	0.07	0.01	20.5
			W: 0.18	0.05	0.04	0.25				0.05	0.04	0.24			
	LC-MS (U)	0.13	**M: 0.20***	(Int: 3.97)	(0.03)	(0.00***)	0.11	0.01	22.2	(Int: 4.08)	(0.08)	(0.00***)	0.04	0.00	22.6
			W: 0.13	0.18	0.11	0.11				0.10	0.12	0.40			
Proline	LC-MS (U)	-0.05	M: 0.03	(Int: 3.97)	(0.03)	(0.00***)	0.24	0.06	22.3	(Int: 4.10)	(0.07)	(0.00***)	0.04	0.00	22.6
			W: 0.05	0.06	0.07	0.40				0.07	0.07	0.32			
	GC-MS (P)	**-0.16***	M: -0.15	(Int: 3.9)	(0.03)	(0.00***)	0.05	0.00	17.1	(Int: 3.98)	(0.07)	(0.00***)	0.01	0.00	17.7
			W: -0.11	-0.07	0.04	0.07				-0.07	0.04	0.10			
Alanine	GC-MS (U)	0.12	M: 0.04	(Int: 3.97)	(0.03)	(0.00***)	-0.22	0.05	18.9	(Int: 4.10)	(0.07)	(0.00***)	0.07	0.00	18.5
			W: -0.14	0.00	0.05	0.96				0.02	0.05	0.73			
Pyroglutamate	GC-MS (U)	-0.01	M: -0.06	(Int: 3.97)	(0.03)	(0.00***)	0.11	0.01	18.4	(Int: 4.09)	(0.07)	(0.00***)	0.26	0.07	18.2
			W: -0.09	-0.12	0.10	0.24				-0.08	0.10	0.45			
Methionine	GC-MS (P)	-0.14	M: -0.10	(Int: 3.91)	(0.03)	(0.00***)	-0.02	0.00	17.3	(Int: 3.98)	(0.07)	(0.00***)	-0.08	0.01	17.8
			W: -0.16	-0.13	0.08	0.08				-0.13	0.08	0.10			
Leucine	GC-MS (P)	-0.11	M: -0.03	(Int: 3.91)	(0.03)	(0.00***)	-0.09	0.01	16.7	(Int: 3.98)	(0.07)	(0.00***)	-0.04	0.00	17.1
			W: -0.19	-0.14	0.10	0.15				-0.14	0.11	0.19			
Glutamic acid	GC-MS (P)	-0.04	M: 0.00	(Int: 3.91)	(0.03)	(0.00***)	-0.01	0.00	16.3	(Int: 3.99)	(0.07)	(0.00***)	-0.02	0.00	16.8
			W: -0.05	-0.02	0.05	0.77				-0.01	0.05	0.81			
Valine	GC-MS (P)	-0.12	M: -0.08	(Int: 3.91)	(0.03)	(0.00***)	-0.05	0.00	17.0	(Int: 3.98)	(0.07)	(0.00***)	-0.07	0.01	17.5
			W: -0.13	-0.13	0.08	0.13				-0.12	0.09	0.16			
Isoleucine	GC-MS (P)	-0.12	M: -0.06	(Int: 3.91)	(0.03)	(0.00***)	-0.10	0.01	17.2	(Int: 3.97)	(0.07)	(0.00***)	-0.07	0.00	17.5
			W: -0.20	-0.14	0.08	0.08				-0.13	0.08	0.12			

C15, pentadecanoic; C17, heptadecanoic acid; FIB, food intake biomarker; GC-MS, gas chromatography mass spectrometry; GLM, generalized linear model; LC-MS, liquid chromatography mass spectrometry; M, men; MAE, mean absolute error; P, plasma; r_{ap}, correlation between actual and predicted intake; SE, standard error; U, urine; W, women. Significant results are bolded: *$p \leq 0.05$, *** $p \leq 0.001$.

[a] For the current study, biomarkers that were previously detected in serum were validated in plasma. For biomarkers that were previously detected using NMR, GC-MS was used as a substitution platform. A few biomarkers were not visible (aminoadipic acid (LC-MS plasma and urine), citrulline (LC-MS plasma), valyl-threonine (LC-MS plasma)) and were therefore not included in the current validation.

[b] Intercept (Int) values for the models are provided in brackets.

[c] Adjusted for age, sex, and BMI.

Assessment of biomarkers for yoghurt intake

Out of the ten candidate FIBs that were previously-identified for yogurt intake in plasma (**Table S2**), eight were detected in plasma in the current study (**Table 5**). No significant differences were found between plasma levels of these FIBs by increasing quintiles of yogurt intake in the total population. However, when stratified into sex-specific intake quintiles, a significant difference was found for plasma tyrosine in women (Q2-5 vs Q1, $p \leq 0.05$), although it should be noted that Q1 comprised primarily non-consumers (**Figure S5**).

Spearman's correlations were weak and non-significant for all FIBs. Similarly, there were no significant single-marker models for yoghurt (unadjusted or adjusted) (**Table 5**). From the multi-marker models, a significant adjusted model consisting of threonine + tyrosine + sex was generated for yogurt intake (**Table S9**). However, the model performance was very poor ($r_{ap} = 0.03$, $R^2 = 0.0008$, MAE = 68 g/d).

Table 5. Single-marker validation results for previously-identified candidate FIBs for yogurt

Biomarker	Analytical Platform (Biosample)[a]	Spearman's correlation coefficient (r_s)		Unadjusted GLM[b]						Adjusted GLM[b,c]					
				Coefficient	SE	*p*-value	r_{ap}	R^2	MAE	Coefficient	SE	*p*-value	r_{ap}	R^2	MAE
Proline	LC-MS (P)	0.01	M: 0.01	(Int: 4.53)	(0.06)	(0.00***)	0.13	0.02	68.0	(Int: 4.69)	(0.13)	(0.00***)	-0.12	0.02	68.5
			W: 0.17	-0.01	0.06	0.89				0.01	0.06	0.92			
Indole-3-lactic acid	LC-MS (P)	0.03	M: 0.01	(Int: 4.53)	(0.06)	(0.00***)	-0.05	0.00	67.8	(Int: 4.68)	(0.13)	(0.00***)	-0.15	0.02	68.7
			W: 0.14	0.02	0.08	0.80				0.03	0.08	0.73			
Lysine	LC-MS (P)	0.02	M: -0.02	(Int: 4.53)	(0.06)	(0.00***)	0.08	0.01	67.8	(Int: 4.69)	(0.13)	(0.00***)	-0.16	0.03	68.5
			W: 0.20	0.01	0.07	0.89				0.02	0.07	0.81			
Threonine	LC-MS (P)	0.04	M: -0.01	(Int: 4.53)	(0.06)	(0.00***)	0.02	0.00	67.9	(Int: 4.68)	(0.13)	(0.00***)	-0.13	0.02	68.5
			W: 0.20	-0.01	0.06	0.92				-0.00	0.06	0.97			
Phenylalanine	LC-MS (P)	0.08	M: 0.07	(Int: 4.53)	(0.06)	(0.00***)	0.01	0.00	67.6	(Int: 4.69)	(0.13)	(0.00***)	-0.12	0.01	68.3
			W: 0.17	0.03	0.06	0.64				0.04	0.06	0.53			
Tyrosine	LC-MS (P)	0.12	M: 0.10	(Int: 4.52)	(0.06)	(0.00***)	-0.09	0.01	67.4	(Int: 4.70)	(0.13)	(0.00***)	-0.15	0.02	68.1
			W: 0.21	0.06	0.06	0.29				0.07	0.06	0.21			
Tryptophan	LC-MS (P)	0.03	M: 0.02	(Int: 4.53)	(0.06)	(0.00***)	-0.15	0.02	68.0	(Int: 4.69)	(0.13)	(0.00***)	-0.17	0.03	69.0
			W: 0.10	0.02	0.08	0.83				0.02	0.07	0.75			
Indole-3-acetaldehyde	LC-MS (P)	0.03	M: 0.00	(Int: 4.53)	(0.06)	(0.00***)	-0.17	0.03	67.7	(Int: 4.69)	(0.13)	(0.00***)	-0.15	0.02	68.4
			W: 0.15	0.03	0.09	0.70				0.05	0.09	0.59			

FIB, food intake biomarker; GLM, generalized linear model; LC-MS, liquid chromatography mass spectrometry; M, men; MAE, mean absolute error; P, plasma; r_{ap}, correlation between actual and predicted intake; SE, standard error; W, women. Significant results are bolded: *** $p \leq 0.001$.

[a] For the current study, biomarkers that were previously detected in serum were validated in plasma. A few biomarkers were not visible (citrulline (LC-MS plasma) and asparagine (LC-MS plasma)) and were therefore not included in the current validation.

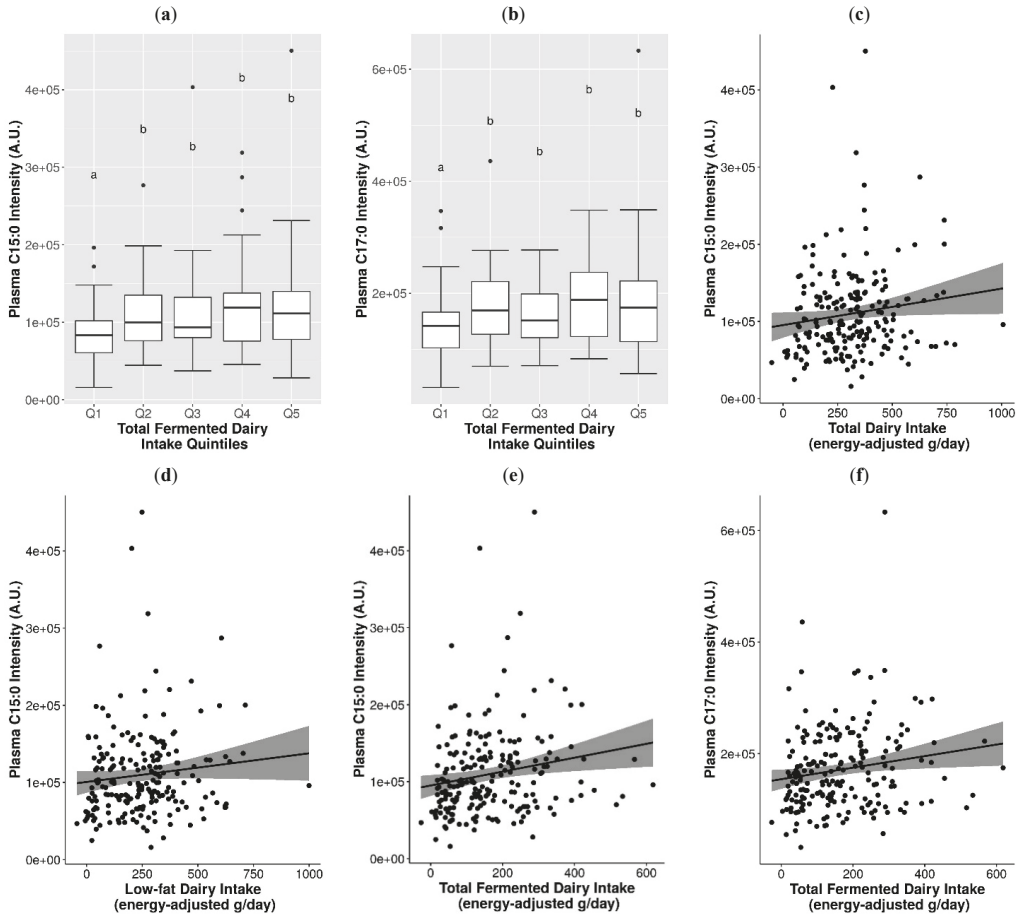
[b] Intercept (Int) values for the models are provided in brackets.

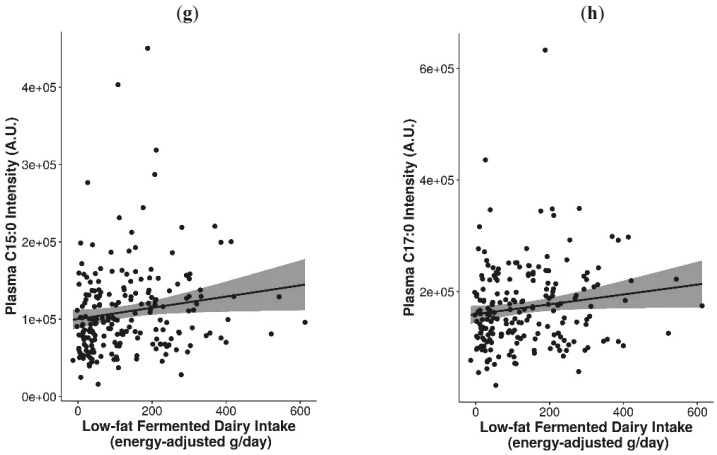
[c] Adjusted for age, sex, and BMI.

Assessment of pentadecanoic acid (C15) and heptadecanoic acid (C17) as biomarkers for general dairy intake

Differences in the relative abundance of C15 and C17 in fasting plasma were assessed by quintiles of intake for various dairy groups. Significantly higher C15 and C17 were observed with higher quintiles of total fermented dairy intake (Q2-Q5 vs. Q1, $p \leq 0.05$) (**Figure 2ab**), but not for other dairy groups. For C17 and total non-fermented dairy intake, the effect was not clear (Q1, Q3, Q5 vs Q2, Q4 vs Q3, $p \leq 0.05$) (**Figure S6**). In addition, no significant differences in levels of these fatty acids were observed between intake quintiles for other dairy groups (including total dairy), even when stratified into sex-specific intake quintiles.

Figure 2. Significantly increased plasma pentadecanoic acid (C15) and/or heptadecanoic acid (C17) with increasing dairy intake. (a) C15 by quintiles of total fermented dairy intake, (b) C17 by quintiles of total fermented dairy intake (significance between quintiles denoted by different letters, $p \leq 0.05$), (c) C15 by continuous total dairy intake, (d) C15 by continuous low-fat dairy intake, (e) C15 by continuous total fermented dairy intake, (f) C17 by continuous total fermented dairy intake, (g) C15 by continuous low-fat fermented dairy intake, (h) C17 by continuous low-fat fermented dairy intake.





The seemingly stronger links between these biomarkers and fermented dairy groups was further observed in analyses with continuous intakes. Although correlations between C15 or C17 with dairy groups were generally weak, they were positive and significant for total dairy (C15 $r_s = 0.17$), low-fat dairy (C15 $r_s = 0.16$), total fermented dairy (C15 $r_s = 0.24$; C17 $r_s = 0.19$), and low-fat fermented dairy (C15 $r_s = 0.19$; C17 $r_s = 0.16$) ($p \leq 0.05$) (**Table 6** and **Figure 2c-h**). When stratified by sex, significant positive correlations were observed in men for C15 and C17 with low-fat dairy, total fermented dairy, and between C15 and low-fat fermented dairy. Similarly, in the single-marker regression models, positive and significant models were generated for C15 and total dairy, total fermented dairy, and low-fat fermented dairy intake, and similarly for C17 and total and low-fat fermented dairy intake (**Table 6**). Adjustment of the models by sex, BMI, and age also revealed a significant model for C15 and low-fat dairy. For C15 and total dairy intake, adjustment measurably improved the model performance ($r_s = 0.3$, $R^2 = 0.1$, MAE = 125 g/d).

The best multi-marker models for C15 and C17 derived from stepwise regression for dairy foods and dairy groups are presented in **Table S10**. In the unadjusted models, C15 alone was revealed to be the best predictor of intakes for all dairy groups, and was significant for total dairy, total fermented dairy, and low-fat fermented dairy. In the adjusted models, the best (most parsimonious) models were generated from a combination of biomarker/covariates, and consisted of C15 + sex + BMI for total dairy, low-fat dairy, and total non-fermented dairy, C15 + sex for total fermented dairy, and C15 + age for low-fat fermented dairy. For low-fat non-fermented dairy, the best model did not include a biomarker but was instead driven by two covariates (sex + BMI). C15 was positively associated with dairy intakes in all models, while covariates were negatively associated. In all cases, the adjusted model selected from stepwise regression had the best model outcomes (in terms of model significance and prediction performance); however, this did not involve a true multi-marker combination consisting of C15 + C17. It is noteworthy that a high degree of multicollinearity between C15 and C17 was observed (variance inflation factor (VIF)>5, r = 0.93)).

Table 6. Single-marker validation results for pentadecanoic acid (C15) and heptadecanoic acid (C17) by dairy group

Biomarker	Analytical Platform (Biosample)	Spearman's correlation coefficient (r_s)		Unadjusted GLM[a]						Adjusted GLM[a,b]					
				Coefficient	SE	*p*-value	r_{ap}	R^2	MAE	Coefficient	SE	*p*-value	r_{ap}	R^2	MAE
Total Dairy															
C15	GC-MS (P)	**0.17***	M: 0.17	**(Int: 5.89)**	**(0.03)**	**(0.00***)**	**0.06**	**0.00**	**130.9**	**(Int: 6.08)**	**(0.07)**	**(0.00***)**	**0.31**	**0.10**	**125.4**
			W: 0.13	**0.16**	**0.07**	**0.02***				**0.17**	**0.07**	**0.02***			
C17	GC-MS (P)	0.12	M: 0.14	(Int: 5.89)	(0.03)	(0.00***)	-0.01	0.00	128.1	(Int: 6.08)	(0.07)	(0.00***)	0.37	0.14	122.7
			W: 0.12	0.15	0.08	0.07				0.16	0.08	0.05			
High-fat Dairy															
C15	GC-MS (P)	-0.01	M: -0.04	(Int: 4.22)	(0.06)	(0.00***)	0.03	0.00	56.2	(Int: 4.30)	(0.12)	(0.00***)	0.10	0.01	56.2
			W: 0.09	0.15	0.12	0.21				0.16	0.12	0.20			
C17	GC-MS (P)	-0.06	M: -0.08	(Int: 4.22)	(0.06)	(0.00***)	-0.03	0.00	56.0	(Int: 4.30)	(0.12)	(0.00***)	-0.01	0.00	56.1
			W: 0.00	0.10	0.14	0.49				0.10	0.14	0.50			
Low-fat Dairy															
C15	GC-MS (P)	**0.16***	**M: 0.19***	(Int: 5.68)	(0.04)	(0.00***)	0.07	0.01	139.5	**(Int: 5.89)**	**(0.08)**	**(0.00***)**	**0.26**	**0.07**	**136.6**
			W: 0.07	0.16	0.09	0.06				**0.17**	**0.09**	**0.05***			
C17	GC-MS (P)	0.13	**M: 0.17***	(Int: 5.68)	(0.04)	(0.00***)	0.04	0.00	137.9	(Int: 5.89)	(0.08)	(0.00***)	0.32	0.10	133.8
			W: 0.07	0.16	0.10	0.11				0.17	0.10	0.09			
Total Fermented Dairy															
C15	GC-MS (P)	**0.24*****	**M: 0.24****	**(Int: 5.25)**	**(0.04)**	**(0.00***)**	**0.01**	**0.00**	**107.3**	**(Int: 5.43)**	**(0.09)**	**(0.00***)**	**0.09**	**0.01**	**106.7**
			W: 0.21	**0.27**	**0.09**	**0.00***				**0.25**	**0.09**	**0.01****			
C17	GC-MS (P)	**0.19****	**M: 0.20***	**(Int: 5.25)**	**(0.04)**	**(0.00***)**	**-0.04**	**0.00**	**105.3**	**(Int: 5.43)**	**(0.09)**	**(0.00***)**	**0.06**	**0.00**	**103.7**
			W: 0.18	**0.26**	**0.11**	**0.01***				**0.23**	**0.11**	**0.03***			
High-fat Fermented Dairy															
C15	GC-MS (P)	0.05	M: 0.07	(Int: 3.80)	(0.06)	(0.00***)	0.18	0.03	35.7	(Int: 3.85)	(0.13)	(0.00***)	0.14	0.02	35.9
			W: 0.04	0.20	0.13	0.11				0.24	0.13	0.06			
C17	GC-MS (P)	0.01	M: 0.06	(Int: 3.80)	(0.06)	(0.00***)	0.10	0.01	36.2	(Int: 3.85)	(0.13)	(0.00***)	0.02	0.00	36.4
			W: -0.07	0.14	0.15	0.34				0.17	0.15	0.26			
Low-fat Fermented Dairy															
C15	GC-MS (P)	**0.19****	**M: 0.19***	**(Int: 4.98)**	**(0.06)**	**(0.00***)**	**-0.03**	**0.00**	**101.1**	**(Int: 5.20)**	**(0.11)**	**(0.00***)**	**0.04**	**0.00**	**103.6**
			W: 0.19	**0.29**	**0.12**	**0.01***				**0.25**	**0.12**	**0.03***			
C17	GC-MS (P)	**0.16***	M: 0.15	**(Int: 4.99)**	**(0.06)**	**(0.00***)**	**-0.05**	**0.00**	**97.7**	(Int: 5.20)	(0.11)	(0.00***)	0.04	0.00	99.0
			W: 0.19	**0.30**	**0.14**	**0.03***				0.25	0.13	0.06			
Total Non-fermented Dairy															
C15	GC-MS (P)	0.03	M: 0.06	(Int: 5.14)	(0.05)	(0.00***)	0.12	0.01	87.3	(Int: 5.33)	(0.11)	(0.00***)	0.07	0.01	88.3

			W: 0.02	0.04	0.11	0.74				0.08	0.11	0.48			
C17	GC-MS (P)	0.02	M: 0.06	(Int: 5.14)	(0.05)	(0.00***)	-0.11	0.01	87.2	(Int: 5.33)	(0.11)	(0.00***)	0.09	0.01	88.0
			W: 0.01	0.03	0.13	0.84				0.07	0.13	0.57			
High-fat Non-fermented Dairy															
C15	GC-MS (P)	-0.09	M: -0.12	(Int: 3.71)	(0.06)	(0.00***)	-0.13	0.02	29.5	(Int: 3.79)	(0.13)	(0.00***)	-0.20	0.04	29.9
			W: -0.01	0.03	0.13	0.83				0.00	0.13	1.00			
C17	GC-MS (P)	-0.12	**M: -0.19***	(Int: 3.71)	(0.06)	(0.00***)	-0.09	0.01	29.4	(Int: 3.79)	(0.13)	(0.000***)	-0.18	0.03	29.7
			W: 0.00	0.01	0.15	0.96				-0.02	0.15	0.88			
Low-fat Non-fermented Dairy															
C15	GC-MS (P)	0.03	M: 0.10	(Int: 4.99)	(0.06)	(0.00***)	0.15	0.02	97.1	(Int: 5.19)	(0.12)	(0.00***)	0.19	0.04	95.0
			W: -0.05	0.03	0.13	0.79				0.09	0.13	0.48			
C17	GC-MS (P)	0.03	M: 0.12	(Int: 4.99)	(0.06)	(0.00***)	-0.14	0.02	97.1	(Int: 5.19)	(0.12)	(0.00***)	0.21	0.04	95.0
			W: -0.07	0.03	0.15	0.85				0.09	0.15	0.55			

C15, pentadecanoic; C17, heptadecanoic acid; GC-MS, gas chromatography mass spectrometry; GLM, generalized linear model; M, men; MAE, mean absolute error; P, plasma; r_{ap}, correlation between actual and predicted intake; SE, standard error; W, women. Significant results are bolded: *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

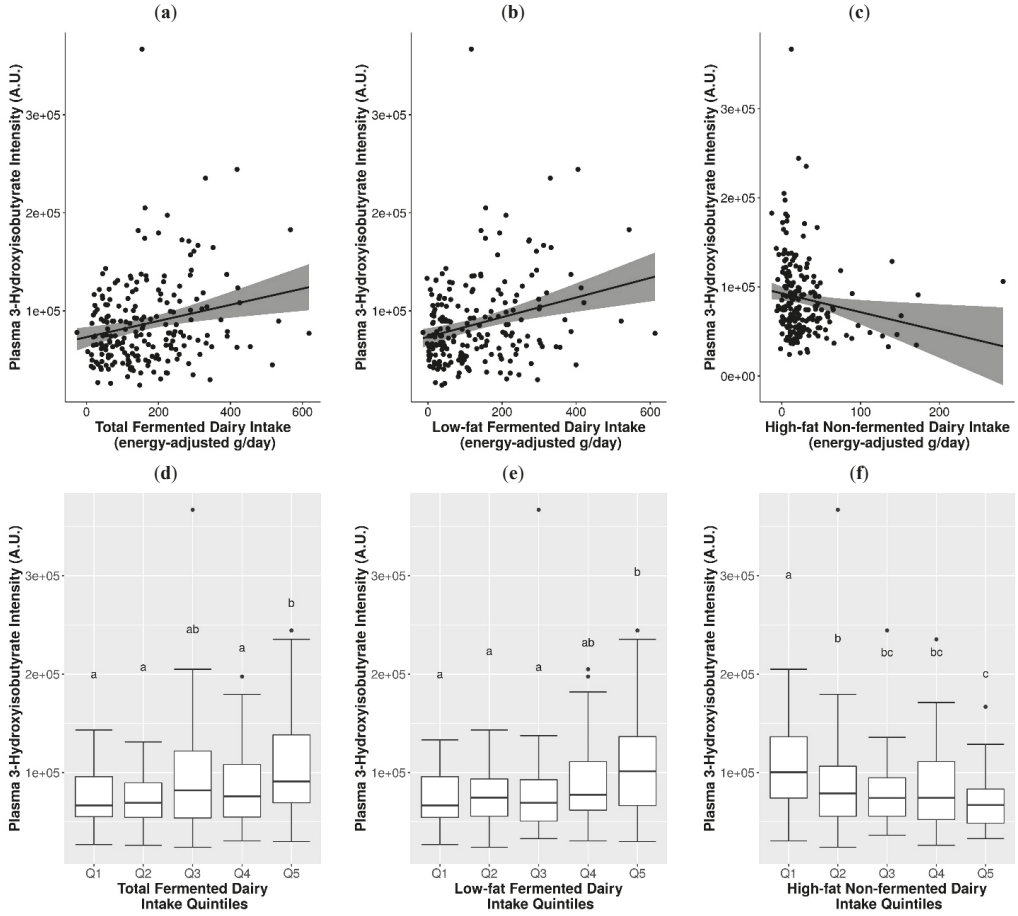
[a] Intercept (Int) values for the models are provided in brackets.

[b] Adjusted for age, sex, and BMI.

Suitability of biomarkers for discriminating between fermented and non-fermented dairy intake

Several of the FIBs had significant positive correlations with fermented dairy intake and/or negative correlations with non-fermented dairy intake (**Table S11**). In particular, consistent correlations were observed for plasma 3-hydroxybutyrate (r_s = 0.2 for total fermented dairy, r_s = 0.23 for low-fat fermented dairy intake, r_s = -0.24 for high-fat non-fermented dairy intake; $p \leq 0.05$) and were reflected in the different levels of 3-hydroxybutyrate for the different quintiles of intake for these dairy groups (**Figure 3**). Other relevant markers included plasma C15, C17, galactonate, lactose, valine, galactitol, 3-phenyllactic acid, glutamic acid, isoleucine, leucine, methionine, and proline (detected with GC-MS) (**Figure 3** and **Figure S7**). These FIBs were included in an exploratory multi-marker model to gauge whether their inclusion can help to better predict fermented and non-fermented dairy intake.

Figure 3. Significant differences in plasma 3-hydroxyisobutyrate levels between fermented and non-fermented dairy groups. Analyzed by continuous intake or intake quintiles of (a,d) total fermented dairy, (b,e) low-fat fermented dairy intake, and (c,f) high-fat non-fermented dairy intake. Significance between quintiles denoted by different letters ($p \leq 0.05$).



As seen in **Table S12**, when compared to **Table S10**, multi-marker models with these FIBs improved the model performance of the majority of dairy groups compared to models with C15 and/or C17. The best multi-marker models selected for total fermented dairy (C15 + galactonate + glutamic acid + lactose + methionine + 3-hydroxyisobutyrate; unadjusted, r_{ap} = 0.22, R^2 = 0.05, MAE = 101 g/d), high-fat fermented

dairy (C15 + 3-hydroxyisobutyrate + BMI; adjusted, r_{ap} = 0.4, R^2 = 0.16, MAE = 35 g/d), and low-fat fermented dairy (C15 + lactose + 3-hydroxyisobutyrate + galactonate + glutamic acid + methionine; unadjusted, r_{ap} = 0.25, R^2 = 0.06, MAE = 93 g/d) all included significant positive associations with C15. Furthermore, 3-hydroxyisobutyrate was selected in the best model for all three fermented dairy groups: positively associated for total (non-significant) and low-fat fermented dairy (significant), and negatively associated with high-fat fermented dairy (significant). The best performing models for non-fermented dairy groups included valine + 3-hydroxyisobutyrate + 3-phenyllactic acid + BMI + sex for total non-fermented dairy (adjusted, r_{ap} = 0.02, R^2 = 0.00, MAE = 93 g/d), C17 + isoleucine + leucine for high-fat non-fermented dairy (unadjusted and adjusted, r_{ap} = 0.4, R^2 = 0.16, MAE = 27 g/d), and valine + 3-hydroxyisobutyrate + 3-phenyllactic acid + sex + BMI for low-fat non-fermented dairy (adjusted, r_{ap} = 0.11, R^2 = 0.01, MAE = 96 g/d). For total and low-fat non-fermented dairy, 3-hydroxyisobutyrate was significantly and negatively associated with intake in adjusted models. Furthermore, inclusion of these candidate FIBs also improved the prediction of high-fat and low-fat dairy groups (r_{ap} = 0.27-0.5, R^2 = 0.07-0.25) (**Table S12**).

Discussion

In the current study, we aimed to evaluate the robustness of the previously-identified candidate FIBs for milk, cheese, and yogurt. Most of the selected biomarkers have already shown some of the essential qualities of a FIB including plausibility and time-response in a controlled intervention setting (18-22), but observational data in free-living populations is limited. The single-marker models examined in this observational study did not perform well in predicting the intake of dairy foods in our free-living population, which may be related to the fact that these FIBs are non-specific and can be influenced by consumption of other foods in the diet. However, we observed modest associations for multi-marker models that also account for known covariates, suggesting that they may help better capture the subtle differences between specific dairy foods. Moreover, our analyses illustrate several challenges and considerations critical to further validation of these FIBs.

Biomarkers for general dairy intake, dairy food intake, and their specificity

By far, the most common dietary biomarkers described in studies of dairy intake are C15 and C17. Despite their widespread use, several limitations have been acknowledged, including their non-specificity for dairy in populations with high fish intake due to their endogenous presence in fish (13). Furthermore, although C15 has been suggested to be an effective concentration biomarker of dairy intake in controlled animal studies, only moderate correlations have been reported in human observational studies (48). Due to these limitations, as well as the inability of these biomarkers to discriminate between specific dairy foods, the identification of further FIBs for dairy products is a valuable endeavour. A previous systematic review on biomarkers of dairy products identified several plausible FIBs of total dairy intake, including serum C15, C17, C17:1, myristoyl-sphingomyelin SM(d18:1/14:0), and galactonate, as well as urinary isovalerylglutamic acid, isovalerylglycine, tiglylglycine, and isobutyrylglycine for cheese intake (49). No specific biomarkers were identified for yogurt consumption.

In the present study, we evaluated the association of C15 and C17 in fasting plasma with dairy intake, the results of which helped contextualize the associations and validation performances of the other FIBs. Although associations were generally low (r_s = 0.16-0.24), they were comparable to observational studies with similar study designs (r = 0.1-0.36) (50-52). Other FIBs we aimed to evaluate for milk, cheese, and yogurt (19-22) were mainly non-significant, or if significant, yielded weak positive associations. This may be partly due to the presence of the FIBs or their parent compounds in different foods. For instance, while lactose is the predominant carbohydrate in milk, its presence in commonly-consumed processed foods containing milk ingredients may obscure the specificity of lactose and its metabolites for assessing milk intake (53).

Additionally, the majority of FIBs for cheese and yogurt (peptides, amino acids, and their intermediates) can also be influenced by the consumption of a large variety of protein-rich foods in the diet. The single-marker validation of these non-specific FIBs in a free-living population presents a tremendous challenge, but their inclusion in a multi-marker panel appears to be more promising.

Single- versus multi-marker models for evaluating the robustness of FIBs

Since milk is a complex mixture of macronutrients, micronutrients, minerals, and bioactive compounds, it is intuitive to seek out multiple biomarkers to capture and discriminate the intake of milk and dairy products. By using regression models, we could assess and compare the ability of single- vs. multi-marker approaches in predicting intake of specific dairy foods and dairy groups. Selected physiological covariates that can affect and/or be affected by the choice of dairy food consumed as well as absolute intake levels and patterns of consumption (sex, age, and BMI) (54) were also included in the biomarker models.

In our models, C15 performed better than C17 for predicting general dairy intake in both single- and multi-marker models, confirming what has been previously observed in the literature. From the single-marker models, urinary lactose, galactose, and galactitol were the most effective FIBs in predicting milk intake (better than C15 or C17), while two adjusted multi-marker models (galactose + galactitol + age + sex + BMI; indole-3-propionic acid + tryptophan + sex) offered slightly improved prediction performance. Galactose, galactitol, and tryptophan were positively associated with milk intake in these models, but indole-3-propionic acid (a deaminated metabolite of tryptophan) was negatively associated with milk intake. While milk consumption previously generated a significant postprandial increase in indole-3-propionic acid (21), it was not detected in milk, suggesting that indole-3-propionic acid may have been synthesized from tryptophan in milk by the gut microbiota (55).

A significant multi-marker model consisting of plasma C15, isoleucine and glutamic acid captured cheese intake, whereas no significant single-marker models were generated. As cheese products tend to be higher in dairy fat (compared to milk and yogurt), it is not surprising that C15 was selected in the multi-marker model for cheese. Similarly, glutamic acid has been previously reported as the primary compound responsible for the ‘umami’ taste quality of cheese products (56). Conversely, isoleucine is ubiquitous in the diet, and cheese consumption may not have been sufficient to impact isoleucine levels significantly. This is reflected in the non-significant but negative correlation between plasma isoleucine and cheese intake, and the negative association in the regression model. For yogurt intake, no significant single-marker models were generated. A significant adjusted multi-marker model comprising threonine and tyrosine was generated for yogurt intake, however, the model performance was low, perhaps due to the non-specific nature of the panel of FIBs for yogurt (primarily amino acids).

Evaluation of other facets of validity

In the case of non-specific biomarkers, a major factor affecting intake-biomarker associations is the quantity of food consumed. The Netherlands has one of the highest per capita dairy consumption, which makes our population highly suitable for evaluating dairy biomarkers. However, within dairy foods, consumption of cheese was comparatively lower than consumption of milk in our population (median ~ 27 vs. 87 g/d), with a narrower range of intakes (8 to 67 g/d vs. 4 to 303 g/d in Q1 to Q5). This can affect the ability of FIBs to discriminate between individuals with high or low intakes, and blurs the dose-response relationship. Although true dose-response could not be evaluated in our study, we observed significant increases in multiple FIBs across quintiles of dairy food intake, in particular, urinary galactitol for milk intake. Several FIBs also showed apparent sex-related responses in the stratified quintile analysis and correlations. These findings could be affected by the differences in numbers of participants between sexes, which may have afforded higher statistical power in men (n ~ 33 per quintile) rather than women (n ~ 16 per quintile).

We also acknowledge that the composition of (bovine) milk can be affected by animal grazing conditions, which could lead to seasonal variations in levels of biomarkers in the blood or urine. A study conducted in the Netherlands reported that the most pronounced differences in milk composition were in fatty acid concentrations (decrease in saturated fatty acids and increase in trans fatty acids during the grazing season, ~April-September), while lactose and protein composition remained relatively stable (57). Similar effects on other metabolites/biomarkers are unknown.

Other validation criteria of reliability, stability, analytical performance, and reproducibility could not be sufficiently addressed here, but a few related considerations are worth noting. For biomarker discovery, the combined use of multiple metabolomics analytical platforms (*e.g.*, LC-MS, GC-MS, and NMR) permits complementary coverage of the metabolome and is particularly valuable for identifying unique sets of FIBs based on individual platform strengths (23). For validation, targeted platforms are often favoured, to quantify a limited panel of compounds but often with improved methodology for a specific compound class. In the case of dairy fatty acids (including C15 and C17), the most widely used methodology for their separation and analysis is a targeted, quantitative method using chromatography-flame ionization detector (GC-FID) (58). Thus, further method development and quantitative analyses of these fatty acids as well as other FIBs for milk, cheese, and yoghurt may improve their performance for estimating dairy (food) intake in a multi-marker model, along with their reliability and analytical performance.

Another important consideration consequential for successful FIB validation is that the choice of biosample may reflect a different time-course associated with intake. Long-term fat intake is best measured using adipose tissue (1-2 years), whereas short-term intake is best assessed using serum phospholipids or cholesteryl esters (past several days) and triglyceride fractions (past several hours) (59-61). In the present study, we used fasting plasma and 24-h urine samples that were banked and readily available for analysis. FIBs with short half-lives in plasma were unsurprisingly not significant. For example, in the metabolomics study of yogurt intake, significant increases in several compounds were observed in postprandial plasma, but almost all were not significant in fasting serum after daily yogurt intake for two weeks (21). For metabolites measured in both plasma and urine (*e.g.*, lactose, Blood Group H disaccharide), higher relative abundance was observed in urine samples that were collected over 24 hours whereas levels were almost undetectable in plasma samples collected under fasting conditions. While these FIBs may not be suitable as markers of habitual intake of dairy foods, they may still be valid as markers of short-term or recent intake. Therefore, further exploration of the FIBs outlined in this study in several other independent observational and intervention studies using samples with different time courses would help assess their robustness as short-term biomarkers.

Influence of fat content and fermentation on dairy biomarkers

One objective in the current study was to explore the potential influence of food-related factors on the efficacy of dairy biomarkers, in particular fat content and fermentation status. Our analyses revealed significant positive associations between plasma C15 with total and low-fat dairy intake, and between C15 and C17 with total and low-fat fermented dairy intake, but similar anticipated results were not observed for high-fat dairy groups. One explanation could be the generally lower consumption of high-fat dairy products (Q1: 10 g/d to Q5: 135 g/d) compared to low-fat dairy products (Q1: 43 g/d to Q5: 480 g/d) in our population. While lower intakes inherently translates to lower concentrations of candidate FIBs in biofluids, the group of high-fat dairy foods also tended to include more sporadically consumed products (*e.g.*, cream with hot meal, whipped cream, milk-based ice cream). A combination of these factors can introduce variability and error. Further, there is a small possibility that these fatty acids are enriched in fermented dairy products, as fermentation of milk has been shown to impact the fatty acid profiles of cheese and yogurt products (62).

From our exploratory analyses assessing the suitability of FIBs for discriminating between fermented and non-fermented dairy intake, a significant positive association was found between 3-hydroxyisobutyrate

and total fermented dairy and low-fat fermented dairy intake, and simultaneously inversely associated with high-fat non-fermented dairy as well as high-fat non-fermented intake, suggesting an overall positive association between this FIB and fermented dairy. This association was also partly reflected in the multi-marker models for fermented and non-fermented dairy groups, although not fully confirmed as the direction of association of 3-hydroxyisobutyrate with the various dairy groups presented a complex pattern. 3-Hydroxyisobutyrate is synthesized in the rumen of dairy cattle via the action of butyrate-producing bacteria, and also in ketogenesis as a catabolic product of valine (63). Further studies are needed to strengthen the biological plausibility of this finding, and drive efforts to identify FIBs related to fermentation that will help elucidate the underlying mechanisms for fermented dairy consumption and cardiometabolic health.

Influence of genetic variants on biomarkers of milk intake

Genetic polymorphisms in key enzymes leads to inter-individual variability in the metabolism of a compound, thereby impacting its efficacy and limiting its capacity as a quantitative biomarker. For dairy foods, a dominant mutation in the lactase enzyme (especially LCT-13910 C>T) is critical for lactose metabolism in adulthood (64). The global prevalence of lactase persistence is highly geographically dependent (*e.g.*, <1% in Asia, >90% in Northern Europe) (64), and in our study population comprising primarily Caucasian Dutch adults, the level of lactase persistence was very high (~95%). This resulted in an uneven distribution between LP and LNP individuals (104 vs. 6), and the effects of lactase persistence on the efficacy of lactose metabolites as FIBs of milk intake could not be evaluated in this study with sufficient statistical power. However, in studies involving larger populations or comprising different ethnic populations, the presence of these genetic variants may be magnified, which could affect the predictions/accuracy of these FIBs and warrants careful consideration.

We also previously observed high inter-individual variation in two Lewis system-related oligosaccharides, Lewis A trisaccharide and Blood Group H disaccharide, identified as potential FIBs of (bovine) milk intake (20). In humans, the production of these fucosylated oligosaccharides is determined by expression of the *FUT2* and *FUT3* genes (20). The majority of individuals with functional FUT2 are deemed 'secretors', while those who inherit a homozygous loss-of-function mutation are deemed 'non-secretors' (65). The non-secretor phenotype (~20% of Caucasians) has been associated with higher susceptibility to various gastrointestinal diseases and infections (65-68). Non-secretors with a functional FUT3 enzyme can still express Lewis A antigens, but in rare cases, mutations of both *FUT3* alleles results in the Lewis negative phenotype (6%) (20, 65). In the present study, a comparable prevalence of secretors, non-secretors, and Lewis negative was observed (79, 17, and 4%, respectively). As expected, significantly higher urinary Blood Group H disaccharide was found in FUT2 secretors; an increase was also observed in plasma but was only of borderline significance, presumably due to low overall concentrations. These metabolites were not found to be discriminant for milk intake in our study, which could be attributed to their largely endogenous origin; nonetheless, attention in larger studies will help clarify their classification and impact as FIBs for milk.

Study limitations

There are several limitations worth noting. Firstly, based on the data available, we relied on a window of ±14 days between biosample collection and the completion of an FFQ, which assesses habitual intake of the previous month. This assumes that dietary consumption patterns the day prior to biosample collection were comparable to the reported intakes, but otherwise, would be a source of measurement error. Secondly, since the FIBs were identified as part of a larger non-targeted study, we relied on metabolite relative abundances instead of absolute quantitative data for the validation, which limits the ability for data integration between analytical platforms. Thirdly, like other studies in metabolomics epidemiology, we used pre-existing biosamples from large observational studies that were not originally designed for the purpose of metabolomics analyses, and sample incubation time could influence levels of certain metabolites. Fourthly, the dairy products

administered in the intervention studies where the FIBs were derived from may have a different compositional profile than those consumed in the free-living cohort. In the particular case of cheese, all FIBs were identified following consumption of Swiss Gruyère cheese, whereas consumption of Dutch cheeses (Edam, Gouda) are predominant in the current study population. Finally, we relied on generalized linear and stepwise regression models for comparing single- and multi-marker validation results, and in particular, for determining the predictive ability of the FIBs. Aside from limitations inherent to regression models (*e.g.*, multicollinearity), we acknowledge that these FIBs may perform better in predicting ranked intakes or binary outcomes (*i.e.*, extreme quintiles). Since we wanted to evaluate the robustness of these biomarkers using the full population, we used a continuous approach, which also permitted comparison to previous studies conducted for C15/C17. Further use of quantitative data for the strongest biomarker models will further assess agreement between biomarker-based and subjective reporting methods.

Conclusions

Multi-marker models factoring in several common physiological covariates was better able to capture the intakes of dairy products, including milk and cheese, over single-marker models. For yogurt, prediction of intakes from both single- and multi-marker models were poor due to lack of specificity of the FIBs, or endogenous origin. Further evaluation of these FIBs as short-term biomarkers, quantification of these FIBs, and discovery of new fermentation biomarkers for dairy foods may help to improve estimates of dairy food intake and disentangle the health effects of dairy foods with different properties.

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List of abbreviations

CLR, centered log ratio; FFQ, food frequency questionnaire; FIB, food intake biomarker; FUT2, galactoside 2-alpha-L-fucosyltransferase 2; FUT3, galactoside 2-alpha-L-fucosyltransferase 3; GC-MS, gas chromatography mass spectrometry; GLM, generalized linear models; LC-MS, liquid chromatography mass spectrometry; LNP, lactase non-persistent; LP, lactase persistent; MAE, mean absolute error; NDARD, National Dietary Assessment Reference Database; NMR, nuclear magnetic resonance; NQplus, Nutritional Questionnaire plus; PCA, principal component analysis; qAIC, quasi-Akaike Information Criterion; QC, quality control; VIF, variance inflation factor.

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Supplementary Materials

Table S1. Classification of dairy foods in the NQplus food frequency questionnaire

Dairy Food Item	Fermentation Status	Subgroup	Fat Content[a] (g/100g food)	Fat Classification[b]
Buttermilk	Fermented	Buttermilk	0.2	Skim
Low-fat cheese (20+/30+)	Fermented	Cheese	14.1	Semi-skim
Regular cheese (40+)	Fermented	Cheese	23.9	Semi-skim
Regular cheese (48+)	Fermented	Cheese	30.3	Full fat
Cheese as snack	Fermented	Cheese	28.9	Semi-skim
Cheese with hot meal	Fermented	Cheese	28.9	Semi-skim
Fat luxury cheese	Fermented	Cheese	35.1	Full fat
Less-fat luxury cheese	Fermented	Cheese	22.0	Semi-skim
Unknown cheese	Fermented	Cheese	28.5	Semi-skim
(Fruit) quark with breakfast	Fermented	Quark	2.5	Skim
Full (fruit) yogurt	Fermented	Yogurt	2.8	Full fat
Full yogurt	Fermented	Yogurt	2.8	Full fat
Semi-skim (fruit) yogurt	Fermented	Yogurt	1.5	Semi-skim
Semi-skim yogurt	Fermented	Yogurt	1.8	Semi-skim
Skim (fruit) yogurt	Fermented	Yogurt	0.2	Skim
Skim yogurt	Fermented	Yogurt	0.2	Skim
Unknown yogurt	Fermented	Yogurt	1.8	Semi-skim
Butter	Non-fermented	Butter	81.1	Full fat
Skim Butter	Non-fermented	Butter	37.0	Semi-skim
Coffee cream	Non-fermented	Cream	9.4	Full fat
Cream with hot meal	Non-fermented	Cream	34.2	Full fat
Whipped cream	Non-fermented	Cream	14.8	Full fat
Milk-based ice cream	Non-fermented	Ice cream	12.0	Full fat
Diet coffee milk	Non-fermented	Milk	4.2	Full fat
Full chocolate milk	Non-fermented	Milk	2.8	Full fat
Full milk	Non-fermented	Milk	3.5	Full fat
Full-fat milk with breakfast	Non-fermented	Milk	3.5	Full fat
Regular full milk	Non-fermented	Milk	3.5	Full fat
Regular semi-skim milk	Non-fermented	Milk	1.5	Semi-skim
Semi-skim chocolate milk	Non-fermented	Milk	1.4	Semi-skim
Semi-skim coffee milk	Non-fermented	Milk	4.1	Full fat
Semi-skim milk	Non-fermented	Milk	1.5	Semi-skim
Semi-skim milk with breakfast	Non-fermented	Milk	1.5	Semi-skim
Skim chocolate milk	Non-fermented	Milk	0.5	Skim
Skim milk	Non-fermented	Milk	0.1	Skim
Unknown chocolate milk	Non-fermented	Milk	1.0	Semi-skim
Unknown coffee milk	Non-fermented	Milk	7.3	Full fat
Unknown milk	Non-fermented	Milk	1.4	Semi-skim
Milk powder for coffee	Non-fermented	Milk, powder	32.3	Full fat

[a] The fat content (g/100g) for all dairy products was determined based on values reported in the Dutch Food Composition Table 2011 (Available from: https://nevo-online.rivm.nl/).

[b] Fat classification was based on the guidelines set by the Dutch Dairy Commodities Act (Overheid.nl. Warenwetbesluit Zuivel), where full-fat dairy included milk and milk products with a fat content >1.80%, cheeses with a fat content ≥50%, and curd cheese/quark and cream cheese with a fat content ≥35%, semi-skim dairy included milk and milk products with a fat content ≥1.50% to ≤1.80%, cheeses with a fat content >10% to <50%, and curd cheese/quark and cream cheese with a fat content ≥10% to ≤34%, and skim dairy included milk and milk products with a fat content ≤0.5%, cheeses with a fat content ≤10%, and curd cheese/quark and cream cheese with a fat content <10%. Additional qualifiers for determining the fat content of Dutch cheeses (based on fat content dry matter) included: full-fat cheese (45+ to 60+), semi-skim cheese (10+ to 40+), and skim cheese (≤10).

Table S2. Previously-identified candidate FIBs for milk, cheese and yoghurt with their platforms and biosamples of detection

Biomarker	Analytical Platform	Biosample[a]	Reference
Milk			
Pentadecanoic acid (C15)	GC-MS	Serum	(1)
Heptadecanoic acid (C17)	GC-MS	Serum	(1)
Phenylalanine	LC-MS	Serum	(2)
Asparagine	LC-MS	Serum	(2)
Tyrosine	LC-MS	Serum	(2)
Tryptophan	LC-MS	Serum	(2)
Taurine	LC-MS	Serum	(2)
Indole-3-propionic acid	LC-MS	Serum	(2)
Indole-3-acetic acid	LC-MS	Serum	(2)
Lactose	GC-MS	Urine	(3)
	GC-MS	Serum	(1)
	NMR	Urine	(3)
Galactose	GC-MS	Urine	(3)
	GC-MS	Serum	(1)
	NMR	Urine	(3)
Galactono-1,5-lactone	GC-MS	Urine	(3)
	GC-MS	Serum	(1)
Galacitol	GC-MS	Urine	(3)
	GC-MS	Serum	(1)
Galactonate	LC-MS	Serum	(4)
	LC-MS	Urine	(4)
	NMR	Urine	(3)
	GC-MS	Serum	(1)
Gluconic acid	LC-MS	Serum	(2)
Delta-Gluconolactone	LC-MS	Serum	(2)
Blood group H disaccharide	LC-MS	Serum	(4)
	LC-MS	Urine	(4)
Lewis A trisaccharide	LC-MS	Serum	(4)
Allantoin	NMR	Urine	(3)
Hippurate	NMR	Urine	(3)
Methionine	NMR	Serum	(1)
Cheese			
Pentadecanoic acid (C15)	GC-MS	Serum	(1)
Heptadecanoic acid (C17)	GC-MS	Serum	(1)
3-Phenyllactic acid	GC-MS	Urine	(3)
	GC-MS	Serum	(1)
3-Hydroxyisobutyrate	NMR	Serum	(1)
Aminoadipic acid	LC-MS	Serum	(4)
	LC-MS	Urine	(4)
Citrulline	LC-MS	Serum	(4)
Valyl-threonine	LC-MS	Serum	(4)
Phenylalanyl-proline	LC-MS	Serum	(4)
	LC-MS	Urine	(4)
Indole-3-lactic acid	LC-MS	Serum	(4)

	LC-MS	Urine	(4)
Proline	LC-MS	Serum	(4)
	NMR	Urine	(3)
	GC-MS	Serum	(1)
Alanine	GC-MS	Urine	(3)
	NMR	Urine	(3)
Pyroglutamate	NMR	Urine	(3)
Methionine	GC-MS	Serum	(1)
	NMR	Serum	(1)
Leucine	GC-MS	Serum	(1)
Glutamic acid	GC-MS	Serum	(1)
Valine+isoleucine	NMR	Serum	(1)
Yoghurt			
Proline	LC-MS	Serum	(2)
Indole-3-lactic acid	LC-MS	Serum	(2)
Citrulline	LC-MS	Serum	(2)
Lysine	LC-MS	Serum	(2)
Threonine	LC-MS	Serum	(2)
Phenylalanine	LC-MS	Serum	(2)
Asparagine	LC-MS	Serum	(2)
Tyrosine	LC-MS	Serum	(2)
Tryptophan	LC-MS	Serum	(2)
Indole-3-acetaldehyde	LC-MS	Serum	(2)

FIB, food intake biomarker; GC-MS, gas chromatography mass spectrometry; LC-MS, liquid chromatography mass spectrometry; NMR, nuclear magnetic resonance.

Table S2 References

1. Trimigno A, Munger L, Picone G, Freiburghaus C, Pimentel G, Vionnet N, et al. GC-MS Based Metabolomics and NMR Spectroscopy Investigation of Food Intake Biomarkers for Milk and Cheese in Serum of Healthy Humans. Metabolites. 2018;8(2).
2. Pimentel G, Burton KJ, von Ah U, Butikofer U, Pralong FP, Vionnet N, et al. Metabolic Footprinting of Fermented Milk Consumption in Serum of Healthy Men. J Nutr. 2018;148(6):851-60.
3. Munger LH, Trimigno A, Picone G, Freiburghaus C, Pimentel G, Burton KJ, et al. Identification of Urinary Food Intake Biomarkers for Milk, Cheese, and Soy-Based Drink by Untargeted GC-MS and NMR in Healthy Humans. J Proteome Res. 2017;16(9):3321-35.
4. Pimentel G, Burnand D, Munger LH, Pralong FP, Vionnet N, Portmann R, et al. Identification of Milk and Cheese Intake Biomarkers in Healthy Adults Reveals High Interindividual Variability of Lewis System-Related Oligosaccharides. J Nutr. 2020;150(5):1058-67.

Table S3. List of suppliers of analytical standards	
Compound	**Supplier**
3-Hydroxyisobutyrate	Sigma-Aldrich, Switzerland, ≥96.0% (sodium salt)
L-(−)-3-Phenyllactic acid	Sigma-Aldrich, Switzerland, ≥99.0%
L-Alanine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Allantoin	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Alpha-aminoadipate	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Asparagine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Blood group H disaccharide	Carbosynth, Compton, Newbury, UK
Citrulline	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Delta-Gluconolactone	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Galactitol	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Galactonate	Sigma-Aldrich, Switzerland, ≥98.0%
Galactono-1,5-lactone	Sigma-Aldrich, Switzerland, ≥99.0% (forms 3 derivatives in solution)
D-Galactose	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Gluconic acid	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Glutamic acid	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Heptadecanoic acid (C17)	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Hippurate	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Indole-3-acetaldehyde	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Indole-3-acetic acid	Sigma-Aldrich, Switzerland, ≥98.0%
Indole-3-lactic acid	Sigma-Aldrich Chemie GmbH (Buchs, Switzerland)
Indole-3-propionic acid	Sigma-Aldrich Chemie GmbH (Buchs, Switzerland)
D-Lactose	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Leucine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Lewis A trisaccharide	Carbosynth, Compton, Newbury, UK
L-Lysine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Methionine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Pentadecanoic acid (C15)	Sigma-Aldrich, Switzerland, ≥99.0%
L-Phenylalanine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Phenylalanyl-proline	Synpeptide Co Ltd, Shanghai, China
L-Proline	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Pyroglutamate	Synpeptide Co Ltd, Shanghai, China
Taurine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Threonine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Tryptophan	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Tyrosine	KitMSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Valine+isoleucine	Synpeptide Co Ltd, Shanghai, China
Valyl-threonine	Synpeptide Co Ltd, Shanghai, China

Table S4. Identification features of compounds analyzed by LC-MS

Identification	Biosample	RT (min)	m/z	Adducts	Measured neutral mass (Da)	Theoretical neutral mass (Da)
Phenylalanine	Plasma	3.23	166.0858	M+H, 2M+H	165.0788	165.07898
Tyrosine	Plasma	2.08	182.0804	M+H	181.0734	181.07389
Tryptophan	Plasma	4.07	205.0968	M+H, M+Na, 2M+Na, 2M+H	204.0898	204.08988
Indole-3-propionic acid	Plasma	8.03	190.0865	M+H, M+Na, M+H-H2O	-	189.07898
Indole-3-acetic acid	Plasma	7.1	176.0698	M+H	175.0628	175.06333
Blood group H disaccharide	Plasma	0.92	349.1113	M+Na	-	326.1213
Lewis A trisaccharide	Plasma	7.3	512.2018	M+H-H2O	-	529.20067
Phenylalanyl-proline	Plasma	4.94	263.1386	M+H	-	262.13174
Indole-3-lactic acid	Plasma	6.37	206.0811	M+H, M+Na	205.0741	205.07389
Proline	Plasma	1.04	116.0704	M+H	115.0634	115.06333
Lysine	Plasma	0.81	147.1125	M+H	146.1055	146.10553
Threonine	Plasma	0.93	164.029	M+2Na-H	-	119.05824
Indole-3-acetaldehyde	Plasma	4.07	160.0754	M+H	-	159.06841
Galactonate	Urine	0.97	219.048069	M+Na	196.0583	196.0583
Blood group H disaccharide	Urine	1.19	349.1101	M+Na, M+H	-	326.1213
Phenylalanyl-proline	Urine	4.98	263.1383	M+H	-	262.13174
Indole-3-lactic acid	Urine	6.4	206.081	M+H	205.074	205.07389
Proline	Urine	1.54	116.0704	M+H	-	115.06333

RT, retention time.

Table S5. Identification features of compounds analyzed by GC-MS								
Compound	**Biosample**	**RT (min)**	**Quantifier Ion**	**Qualifier Ion**	**Ratio (Quant/Qual)**	**RSD QC**	**RI sample**	**RI reference**
3-Hydroxyisobutyrate 2TMS	Plasma	16.71	177	218	50	40.8	1150	1151
3-Phenyllactic acid 2TMS	Plasma	26.48	193	267	14	35.9	1579	1580
Galactitol 6TMS	Plasma	30.83	307	319	120	40.5	1921	1929
Galactonate 6TMS	Plasma	31.4	292	319/333	65/35	69.0	1976	1981
Galactose 5TMS 1MEOXb	Plasma	30.58	319	160	40	23.0	1898	1898
Glutamic acid 3TMS	Plasma	26.9	246	348	8	62.8	1606	1604
Heptadecanoic acid 1TMS	Plasma	32.95	327	342	13	33.3	2136	2138
Isoleucine 2TMS	Plasma	20.5	158	218	22	44.6	1285	1285
Lactose 8TMS 1MEOXa	Plasma	38.01	361	319	50	435.3	2660	2671
Leucine 2TMS	Plasma	19.89	158	232	4.5	28.1	1263	1264
Methionine 2TMS	Plasma	25.4	176	293	10	51.5	1513	1514
Pentadecanoic acid 1TMS	Plasma	31.02	299	314	8	34.7	1939	1942
Proline 2TMS	Plasma	20.72	142	216	5	79.7	1293	1289
Valine 2TMS	Plasma	18.33	144	218	20	44.4	1207	1209
3-Phenyllactic acid 2TMS	Urine	26.44	193	267	14	29.9	1580	1580
Alanine 3TMS	Urine	22.19	188	262	17	53.2	1356	1356
Galactitol 6TMS	Urine	30.79	307	319	175	20.9	1922	1929
Galactonate 6TMS	Urine	31.36	292	319/333	65/35	57.5	1977	1981
Galactose 5TMS 1MEOXb	Urine	30.53	319	160	30	17.5	1898	1898
Hippurate 1TMS	Urine	29.99	105	236	20	18.6	1850	1845
Lactose 8TMS 1MEOXa	Urine	37.95	361	319	50	25.4	2662	2671
Pyroglutamate 2TMS	Urine	25.43	156	258	12	26.5	1518	1518

MEOX, Methoxyamine; QC, quality control; RI, Kovats retention index; RT, retention time; RSD, relative standard deviation; TMS, trimethylsilyl.

Table S6. Quintiles of intake for dairy groups and dairy foods in men (*n* = 165)

Food Group	Median Energy-Adjusted Intakes in g/day									
	n_c	Q1 (*n* = 33)	n_c	Q2 (*n* = 33)	n_c	Q3 (*n* = 33)	n_c	Q4 (*n* = 33)	n_c	Q5 (*n* = 33)
Total dairy	33	109 (801, 132)	33	216 (188, 237)	33	289 (270, 316)	33	359 (349, 383)	33	514 (465, 613)
High-fat dairy	32	12 (7, 16)	33	26 (22, 29)	33	44 (37, 51)	33	74 (65, 84)	33	138 (113, 176)
Low-fat dairy	33	39 (15, 55)	33	140 (118, 167)	33	237 (215, 254)	33	313 (298, 327)	33	472 (390, 597)
Total fermented dairy	33	47 (24, 59)	33	94 (76, 114)	33	143 (134, 156)	33	226 (205, 242)	33	329 (293, 355)
High-fat fermented dairy	7	2 (-1, 5)	33	10 (8, 11)	33	18 (16, 21)	33	35 (28, 46)	33	83 (67, 108)
Low-fat fermented dairy	32	16 (6, 24)	33	53 (43, 64)	33	110 (102, 122)	33	197 (157, 213)	33	304 (268, 348)
Total non-fermented dairy	32	15 (6, 23)	33	53 (42, 64)	33	102 (94, 114)	33	173 (159, 201)	33	300 (274, 360)
High-fat non-fermented dairy	27	4 (1, 6)	33	11 (10, 13)	33	19 (16, 21)	33	36 (30, 42)	33	62 (51, 91)
Low-fat non-fermented dairy	0	-3 (-8, 2)	19	17 (14, 23)	33	65 (53, 82)	33	137 (122, 153)	33	284 (255, 355)
Cheese	32	8 (4, 12)	33	19 (17, 23)	33	29 (27, 32)	33	45 (40, 49)	33	68 (62, 94)
Yogurt	9	0 (-3, 5)	33	41 (27, 52)	33	88 (73, 94)	33	133 (109, 138)	33	189 (151, 208)
Milk	11	4 (-5, 11)	33	39 (30, 49)	33	82 (73, 96)	33	157 (142, 191)	33	287 (259, 355)

FFQ, food frequency questionnaire; n_c, number of consumers. Values are reported as median (IQR), unless otherwise specified.

Table S7. Quintiles of intake for dairy groups and dairy foods in women (*n* = 81)

Food Group	Median Energy-Adjusted Intakes in g/day									
	n_c	Q1 (*n* = 17)	n_c	Q2 (*n* = 16)	n_c	Q3 (*n* = 16)	n_c	Q4 (*n* = 16)	n_c	Q5 (*n* = 16)
Total dairy	17	87 (67, 115)	16	228 (207, 241)	16	338 (316, 344)	16	388 (370, 408)	16	545 (496, 608)
High-fat dairy	15	5 (3, 11)	16	23 (20, 26)	16	36 (32, 47)	16	67 (62, 67)	16	126 (107, 150)
Low-fat dairy	16	5 (29, 72)	16	160 (143, 189)	16	258 (242, 265)	16	338 (322, 349)	16	521 (449, 577)
Total fermented dairy	16	31 (16, 36)	16	84 (57, 93)	16	156 (137, 170)	16	216 (198, 228)	16	366 (317, 424)
High-fat fermented dairy	2	0 (-2, 2)	16	8 (6, 10)	16	16 (14, 21)	16	36 (29, 41)	16	80 (58, 120)
Low-fat fermented dairy	16	14 (9, 23)	16	39 (34, 52)	16	109 (90, 123)	16	189 (166, 203)	16	302 (275, 397)
Total non-fermented dairy	16	17 (-8, 40)	16	56 (52, 73)	16	119 (109, 128)	16	190 (163, 219)	16	345 (311, 375)
High-fat non-fermented dairy	14	1 (-2, 3)	16	9 (7, 10)	16	14 (13, 17)	16	22 (19, 27)	16	55 (38, 77)
Low-fat non-fermented dairy	0	6 (-16, 12)	15	39 (36, 43)	16	102 (67, 112)	16	159 (141, 182)	16	334 (292, 366)
Cheese	14	9 (5, 12)	16	16 (15, 17)	16	22 (21, 30)	16	39 (35, 42)	16	70 (55, 80)
Yogurt	7	9 (3, 12)	16	26 (21, 39)	16	71 (63, 84)	16	124 (105, 135)	16	191 (155, 290)
Milk	2	11 (-10, 20)	16	43 (40, 51)	16	105 (96, 116)	16	168 (151, 196)	16	334 (295, 362)

FFQ, food frequency questionnaire; n_c, number of consumers. Values are reported as median (IQR), unless otherwise specified.

Table S8. Multi-marker validation results for previously-identified candidate FIBs for milk, cheese, and yogurt (Unadjusted Models)[a]

Analytical Platform	Biosample	Biomarker[b]		qAIC	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
Milk											
GC-MS	(P)	C15		205.2	(Int: 5.05) Galactonate: 0.07	(0.06) 0.08	(87.82) 0.92	(0.00***) 0.36	0.13	0.02	87.8
		C17									
		Lactose									
		Galactose									
		Galactitol									
		Methionine									
		Galactonate	x								
	(U)	Lactose		221.7	**(Int: 5.12)** **Galactitol: 0.21**	**(0.05)** **0.10**	**(103.53)** **2.18**	**(0.00***)** **0.03***	**0.17**	**0.03**	**93.6**
		Galactose									
		Galactitol	x								
		Hippurate									
		Galactonate									
LC-MS	(P)	Phenylalanine	x	210.0	(Int: 5.06) Phenylalanine: 0.08	(0.05 0.05	(94.39) 1.64	(0.00***) 0.10	0.25	0.06	104.0
		Tyrosine									
		Tryptophan									
		Indole-3-propionic acid (IPA)									
		Indole-3-acetic acid									
		Lewis A disaccharide									
		Blood Group H trisaccharide									
	(U)	Blood Group H trisaccharide		218.9	(Int: 5.12) Galactonate: 0.04	(0.05) 0.05	(102.30) 0.92	(0.00***) 0.36	0.12	0.01	96.7
		Galactonate	x								
Cheese											
GC-MS	(P)	C15	x (VIF=1.3)	294.4	**(Int: 3.91)** **C15: 0.21** **Isoleucine: -0.34** **Glutamic acid: 0.12**	**(0.03)** **0.07** **0.11** **0.07**	**(116.50)** **2.78** **-3.03** **1.79**	**(0.00***)** **0.01**** **0.00**** **0.08**	**0.16**	**0.03**	**16.8**
		C17									
		3-Phenyllactic acid									
		3-Hydroxyisobutyrate (HIB)									
		Proline									
		Methionine									
		Leucine									
		Glutamic acid	x (VIF=1.9)								

Table S8. Multi-marker validation results for previously-identified candidate FIBs for milk, cheese, and yogurt (Unadjusted Models)[a]

Analytical Platform	Biosample	Biomarker[b]		qAIC	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
LC-MS	(U)	Valine		300.4	(Int: 3.97) Pyroglutamate: -0.12	0.03 0.10	120.99 -1.18	(0.00***) 0.24	0.11	0.01	18.4
		Isoleucine	x (VIF=2.2)								
		3-Phenyllactic acid									
		Alanine									
		Pyroglutamate	x								
	(P)	Indole-3-lactic acid (ILA)		286.2	(Int: 3.88) Proline: 0.06	0.04 0.03	108.94 1.66	(0.00***) 0.10	0.06	0.00	20.2
		Proline	x								
		Phenylalanyl-proline									
	(U)	Indole-3-lactic acid	x	300.0	(Int: 3.97) Indole-3-lactic acid: 0.18	0.03 0.11	120.23 1.62	(0.00***) 0.11	0.11	0.01	22.2
		Phenylalanyl-proline									
		Proline									
Yogurt											
LC-MS	(P)	Proline		217.9	(Int: 4.52) Threonine: -0.15 Tyrosine: 0.17	0.06 0.10 0.09	71.86 -1.51 1.81	(0.00***) 0.13 0.07	0.03	0.00	68.5
		Indole-3-lactic acid									
		Lysine									
		Threonine	x (VIF=2.6)								
		Phenylalanine									
		Tyrosine	x (VIF=2.6)								
		Tryptophan									
		Indole-3-acetaldehyde									

C15, pentadecanoic acid; C17, heptadecanoic acid; FIB, food intake biomarker; GC-MS, gas chromatography mass spectrometry; MAE, mean absolute error; NA, not applicable; P, plasma; qAIC, quasi-Akaike Information Criterion; r_{ap} = correlation between actual and predicted intake; SE, standard error; VIF, variance inflation factor. Significant results are bolded: *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

[a] Intercept (Int) values for the models are provided in brackets.

[b] Biomarkers and/or covariates included in the model are indicated with an 'x'.

Table S9. Multi-marker validation results for previously-identified candidate FIBs for milk, cheese, and yogurt (adjusted models)[a]

Analytical Platform	Biosample	Biomarker[b]		Sex	BMI	Age	qAIC	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
Milk														
GC-MS	(P)	C15		x			206.4	(Int: 5.18) Sex: -0.21	(0.09) 0.12	(57.48) -1.81	(0.00***) 0.07	0.09	0.01	88.4
		C17												
		Lactose												
		Galactose												
		Galactitol												
		Methionine												
		Galactonate												
	(U)	Lactose		x	x	x	224.2	**(Int: 5.31)** **Galactose: 0.06** **Galactitol: 0.18** **Age: 0.17** **Sex: -0.27** **BMI: -0.19**	**(0.11)** **0.03** **0.10** **0.11** **0.11** **0.10**	**(49.69)** **1.83** **1.83** **1.58** **-2.43** **-1.81**	**(0.00***)** **0.07** **0.07** **0.12** **0.02*** **0.07**	**0.20**	**0.04**	**91.8**
		Galactose	x (VIF=1.1)											
		Galactitol	x (VIF=1.0)											
		Hippurate												
		Galactonate												
LC-MS	(P)	Phenylalanine		x			212.4	**(Int: 5.23)** **IPA: -0.15** **Tryptophan: 0.24** **Sex: -0.26**	**(0.09)** **0.09** **0.10** **0.11**	**(60.18)** **-1.70** **2.41** **-2.31**	**(0.00***)** **0.09** **0.02*** **0.02***	**0.25**	**0.06**	**102.8**
		Tyrosine												
		Tryptophan	x (VIF=2.7)											
		Indole-3-propionic acid (IPA)	x (VIF=2.7)											
		Indole-3-acetic acid												
		Lewis A disaccharide												
		Blood Group H trisaccharide												
	(U)	Blood Group H trisaccharide		x			220.0	(Int: 5.25) Sex: -0.20	(0.08) 0.10	63.29) -1.93	(0.00***) 0.06	0.11	0.01	95.6
		Galactonate												
Cheese														
GC-MS	(P)	C15	x (VIF=1.3)				289.4	**(Int: 3.91)** **C15: 0.21** **Isoleucine: -0.34** **Glutamic acid: 0.12**	**(0.03)** **0.08** **0.11** **0.07**	**(116.50)** **2.78** **-3.03** **1.79**	**(0.00***)** **0.01*** **0.00*** **0.08**	**0.16**	**0.03**	**16.8**
		C17												
		3-Phenyllactic acid												
		3-Hydroxyisobutyrate (HIB)												
		Proline												
		Methionine												

Table S9. Multi-marker validation results for previously-identified candidate FIBs for milk, cheese, and yogurt (adjusted models)[a]

Analytical Platform	Biosample	Biomarker[b]		Sex	BMI	Age	qAIC	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
		Leucine												
		Glutamic acid	x (VIF=1.9)											
		Valine												
		Isoleucine	x (VIF=2.2)											
	(U)	3-Phenyllactic acid												
		Alanine				x	301.9	**(Int: 4.01)**	**(0.04)**	**(103.50)**	**(0.00***)**	**0.27**	**0.07**	**18.4**
		Pyroglutamate						**Age: -0.14**	**0.07**	**-2.02**	**0.05***			
LC-MS	(P)	Indole-3-lactic acid (ILA)						(Int: 4.03)	(0.07	(56.31)	(0.00***)			
		Proline	x	x	x		292.0	Proline: 0.07	0.03	1.93	0.06	0.10	0.01	20.3
		Phenylalanyl-proline						Sex: -0.15	0.08	-1.89	0.06			
								BMI: -0.13	0.07	-1.77	0.08			
	(U)	Indole-3-lactic acid												
		Phenylalanyl-proline				x	301.4	**(Int: 4.01)**	**(0.04)**	**(102.74)**	**(0.00***)**	**0.16**	**0.02**	**21.9**
		Proline						**Age: -0.14**	**0.07**	**-1.98**	**0.05***			
Yogurt														
		Proline												
		Indole-3-lactic acid												
		Lysine						**(Int: 4.67)**	**(0.10)**	**(45.61)**	**(0.00***)**			
LC-MS	(P)	Threonine	x (VIF=2.7)	x			221.7	**Threonine: -0.18**	**0.10**	**-1.80**	**0.07**	**0.03**	**0.00**	**68.4**
		Phenylalanine						**Tyrosine: 0.20**	**0.09**	**2.16**	**0.03***			
		Tyrosine	x (VIF=2.8)					**Sex: -0.22**	**0.13**	**-1.72**	**0.09**			
		Tryptophan												
		Indole-3-acetaldehyde												

BMI, body mass index; C15, pentadecanoic acid; C17, heptadecanoic acid; FIB, food intake biomarker; GC-MS, gas chromatography mass spectrometry; MAE, mean absolute error; NA, not applicable; P, plasma; aAIC, quasi-Akaike Information Criterion; rap = correlation between actual and predicted intake; SE, standard error; VIF, variance inflation factor. Significant results are bolded: *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

[a] Intercept (Int) values for the models are provided in brackets.

[b] Biomarkers and/or covariates included in the model are indicated with an 'x'.

Table S10. Multi-marker validation results for pentadecanoic acid (C15) and heptadecanoic acid (C17) by dairy group

	Analytical Platform (Biosample)	Biomarker		Sex	BMI	Age	qAIC	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
Total Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	227.3	**(Int: 5.89)**	**(0.03)**	**(176.558)**	**(0.000***)**	**0.06**	**0.00**	**130.9**
		C17						**C15: 0.16**	**0.07**	**2.318**	**0.021***			
Adjusted[a]	GC-MS (P)	C15	x	x	x		227.9	**(Int: 6.05)**	**(0.06)**	**(94.379)**	**(0.000***)**	**0.31**	**0.10**	**122.5**
		C17						**C15: 0.18**	**0.07**	**2.649**	**0.009***			
								Sex: -0.16	**0.07**	**-2.348**	**0.020***			
								BMI: -0.13	**0.07**	**-1.867**	**0.063**			
High-fat Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	207.2	(Int: 4.22)	(0.06)	(73.557)	(0.000***)	0.03	0.00	56.2
		C17						C15: 0.15	0.12	1.255	0.211			
Adjusted[a]	GC-MS (P)	C15	x				205.2	(Int: 4.22)	(0.06)	(73.557)	(0.000***)	0.03	0.00	56.2
		C17						C15: 0.15	0.12	1.255	0.211			
Low-fat Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	222.5	(Int: 5.68)	(0.04)	(137.717)	(0.000***)	0.07	0.01	139.5
		C17						C15: 0.16	0.09	1.905	0.058			
Adjusted[a]	GC-MS (P)	C15	x	x	x		224.6	**(Int: 5.86)**	**(0.08)**	**(74.310)**	**(0.000***)**	**0.25**	**0.06**	**137.7**
		C17						**C15: 0.19**	**0.09**	**2.201**	**0.029***			
								Sex: -0.19	**0.09**	**-2.208**	**0.028***			
								BMI: -0.13	**0.08**	**-1.564**	**0.119**			
Total Fermented Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	215.0	**(Int: 5.25)**	**(0.04)**	**(121.322)**	**(0.000***)**	**0.01**	**0.00**	**107.3**
		C17						**C15: 0.27**	**0.09**	**3.022**	**0.003****			
Adjusted[a]	GC-MS (P)	C15	x	x			215.7	**(Int: 5.32)**	**(0.05)**	**(105.187)**	**(0.000***)**	**0.07**	**0.01**	**107.3**
		C17						**C15: 0.23**	**0.09**	**2.586**	**0.010***			
								Age: -0.24	**0.10**	**-2.418**	**0.017***			
High-fat Fermented Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	198.9	(Int: 3.80)	(0.06)	(61.54)	(0.00***)	0.18	0.03	35.7
		C17						C15: 0.20	0.13	1.59	0.11			
Adjusted[a]	GC-MS (P)	C15	X (VIF = 9.0)				203.9	(Int: 3.80)	(0.06)	(61.50)	(0.00***)	0.22	0.05	35.6
		C17	x (VIF = 9.0)					C15: 0.68	0.34	1.97	0.05*			
								C17: -0.59	0.40	-1.47	0.14			

Table S10. Multi-marker validation results for pentadecanoic acid (C15) and heptadecanoic acid (C17) by dairy group

	Analytical Platform (Biosample)	Biomarker		Sex	BMI	Age	qAIC	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
Low-fat Fermented Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	207.9	**(Int: 4.98)**	**(0.06)**	**(88.22)**	**(0.00***)**	**-0.03**	**0.00**	**101.1**
		C17						**C15: 0.29**	**0.12**	**2.50**	**0.01***			
Adjusted[a]	GC-MS (P)	C15	x			x	211.4	**(Int: 5.09)**	**(0.06)**	**(79.17)**	**(0.00***)**	**0.03**	**0.00**	**103.6**
		C17						**C15: 0.23**	**0.12**	**2.03**	**0.04***			
								Age: -0.36	**0.13**	**-2.77**	**0.01***			
Total Non-fermented Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	211.2	(Int: 5.14)	(0.05)	(97.24)	(0.00***)	0.12	0.01	87.3
		C17						C15: 0.04	0.11	0.33	0.74			
Adjusted[a]	GC-MS (P)	C15	x	x	x		212.1	**(Int: 5.35)**	**(0.10)**	**(53.74)**	**(0.00***)**	**0.09**	**0.01**	**87.2**
		C17						**BMI: -0.16**	**0.11**	**-1.47**	**0.14**			
								Sex: -0.23	**0.11**	**-2.14**	**0.03***			
High-fat Non-fermented Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	179.8	(Int: 3.71)	(0.06)	(60.83)	(0.00***)	-0.13	0.02	29.5
		C17						C15: 0.03	0.13	0.22	0.83			
Adjusted[a]	GC-MS (P)	C15				x	181.5	(Int: 3.77)	(0.07)	(53.18)	(0.00***)	0.14	0.02	29.1
		C17						Age: -0.19	0.13	-1.44	0.15			
Low-fat Non-fermented Dairy														
Unadjusted[a]	GC-MS (P)	C15	x	NA	NA	NA	203.1	(Int: 4.99)	(0.06)	(81.55)	(0.00***)	0.15	0.02	97.1
		C17						C15: 0.03	0.13	0.27	0.79			
Adjusted[a]	GC-MS (P)	C15		x	x		204.1	**(Int: 5.23)**	**(0.11)**	**(45.73)**	**(0.00***)**	**0.16**	**0.02**	**95.4**
		C17						**BMI: -0.18**	**0.13**	**-1.41**	**0.16**			
								Sex: -0.27	**0.13**	**-2.12**	**0.04***			

BMI, body mass index; C15, pentadecanoic acid; C17, heptadecanoic acid; GC-MS, gas chromatography mass spectrometry; MAE, mean absolute error; NA, not applicable; P, plasma; aAIC, quasi-Akaike Information Criterion; rap = correlation between actual and predicted intake; SE, standard error; VIF, variance inflation factor. Significant results are bolded: *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

[a] Intercept (Int) values for the models are provided in brackets.

[b] Biomarkers and/or covariates included in the model are indicated with an 'x'.

Table S11. Significant Spearman's Correlations for Fermented and Non-Fermented Dairy Groups				
Platform (biosample)	**Dairy Food/Group**	**Compound**	**Spearman's correlation coefficient (r_s)**	***p*-value**
GC-MS (P)	Cheese (fermented)	Proline	-0.160	0.022
GC-MS (P)	High-fat fermented dairy	Galactitol	-0.146	0.038
GC-MS (P)	High-fat fermented dairy	Galactonate	-0.158	0.024
GC-MS (P)	High-fat fermented dairy	Isoleucine	-0.159	0.023
GC-MS (P)	High-fat fermented dairy	Leucine	-0.152	0.030
GC-MS (P)	High-fat fermented dairy	Methionine	-0.160	0.022
GC-MS (P)	High-fat fermented dairy	Proline	-0.208	0.003
GC-MS (P)	High-fat fermented dairy	Valine	-0.178	0.011
GC-MS (P)	High-fat fermented dairy	3-Hydroxyisobutyrate	-0.185	0.008
GC-MS (P)	High-fat fermented dairy	3-Phenyllactic acid	-0.164	0.019
GC-MS (P)	Low-fat fermented dairy	Galactitol	0.145	0.038
GC-MS (P)	Low-fat fermented dairy	Galactonate	0.193	0.006
GC-MS (P)	Low-fat fermented dairy	Heptadecanoic acid (C17)	0.155	0.027
GC-MS (P)	Low-fat fermented dairy	Lactose	0.207	0.003
GC-MS (P)	Low-fat fermented dairy	Pentadecanoic acid (C15)	0.193	0.006
GC-MS (P)	Low-fat fermented dairy	Valine	0.141	0.044
GC-MS (P)	Low-fat fermented dairy	3-Hydroxyisobutyrate	0.229	0.001
GC-MS (P)	High-fat non-fermented dairy	Galactonate	-0.163	0.020
GC-MS (P)	High-fat non-fermented dairy	Glutamic acid	-0.162	0.021
GC-MS (P)	High-fat non-fermented dairy	Isoleucine	-0.269	0.000
GC-MS (P)	High-fat non-fermented dairy	Lactose	-0.145	0.039
GC-MS (P)	High-fat non-fermented dairy	Leucine	-0.152	0.030
GC-MS (P)	High-fat non-fermented dairy	Methionine	-0.236	0.001
GC-MS (P)	High-fat non-fermented dairy	Proline	-0.27	0.000
GC-MS (P)	High-fat non-fermented dairy	Valine	-0.279	0.000
GC-MS (P)	High-fat non-fermented dairy	3-Hydroxyisobutyrate	-0.244	0.000
GC-MS (P)	High-fat non-fermented dairy	3-Phenyllactic acid	-0.198	0.005
GC-MS (P)	Total fermented dairy	Galactitol	0.145	0.039
GC-MS (P)	Total fermented dairy	Galactonate	0.182	0.009
GC-MS (P)	Total fermented dairy	Heptadecanoic acid (C17)	0.187	0.007
GC-MS (P)	Total fermented dairy	Lactose	0.207	0.003
GC-MS (P)	Total fermented dairy	Pentadecanoic acid (C15)	0.236	0.001

Table S11. Significant Spearman's Correlations for Fermented and Non-Fermented Dairy Groups

Platform (biosample)	Dairy Food/Group	Compound	Spearman's correlation coefficient (r_s)	*p*-value
GC-MS (P)	Total fermented dairy	3-Hydroxyisobutyrate	0.204	0.004
GC-MS (P)	Yoghurt (fermented)	Galactitol	0.146	0.037
GC-MS (P)	Yoghurt (fermented)	Galactonate	0.15	0.033
GC-MS (P)	Yoghurt (fermented)	Pentadecanoic acid (C15)	0.165	0.019
GC-MS (U)	Cheese (fermented)	Galactitol	0.221	0.001
GC-MS (U)	Cheese (fermented)	Lactose	0.187	0.006
GC-MS (U)	High-fat non-fermented dairy	Galactonate	0.227	0.001
GC-MS (U)	Low-fat fermented dairy	Galactonate	0.182	0.008
GC-MS (U)	Low-fat fermented dairy	3-Phenyllactic acid	0.223	0.001
GC-MS (U)	Low-fat non-fermented dairy	Galactitol	0.191	0.005
GC-MS (U)	Low-fat non-fermented dairy	Lactose	0.168	0.013
GC-MS (U)	Milk (non-fermented)	Alanine	0.135	0.048
GC-MS (U)	Milk (non-fermented)	Galactitol	0.199	0.003
GC-MS (U)	Milk (non-fermented)	Lactose	0.161	0.018
GC-MS (U)	Total fermented dairy	Galactonate	0.189	0.005
GC-MS (U)	Total fermented dairy	3-Phenyllactic acid	0.188	0.006
GC-MS (U)	Yoghurt (fermented)	Hippurate	0.141	0.038
LC-MS (P)	High-fat non-fermented dairy	Indole-3-acetaldehyde	0.174	0.012
LC-MS (P)	High-fat non-fermented dairy	Tryptophan	0.161	0.021
LC-MS (P)	High-fat non-fermented dairy	Phenylalanine	0.137	0.049
LC-MS (P)	Low-fat fermented dairy	Blood group H disaccharide	0.244	0.000
LC-MS (P)	Milk (non-fermented)	Threonine	0.144	0.039
LC-MS (P)	Total fermented dairy	Blood group H disaccharide	0.256	0.000
LC-MS (P)	Total fermented dairy	Tyrosine	0.158	0.024
LC-MS (P)	Total non-fermented dairy	Indole-3-acetaldehyde	0.153	0.029
LC-MS (P)	Total non-fermented dairy	Tryptophan	0.144	0.039
LC-MS (P)	Total non-fermented dairy	Lysine	0.138	0.048
LC-MS (P)	Total non-fermented dairy	Threonine	0.16	0.022
LC-MS (P)	Yoghurt (fermented)	Blood group H disaccharide	0.203	0.003
LC-MS (U)	Total non-fermented dairy	Galactonate	0.149	0.049

C15, pentadecanoic acid; C17, heptadecanoic acid; GC-MS, gas chromatography mass spectrometry; LC-MS, liquid chromatography mass spectrometry; P, plasma; U, urine.
Significant indicated as: *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table S12. Multi-marker validation results for FIBs differentiating between fermented and non-fermented dairy intake by dairy group										
	Analytical Platform (Biosample)	Biomarker	VIF	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
Total dairy										
Unadjusted[a]	GC-MS (P)	(Int)	NA	(5.87)	(0.04)	(156.44)	(0.00***)	0.24	0.06	129.2
		C15	1.4	0.19	0.10	1.98	0.05*			
		Lactose	1.4	0.18	0.06	3.04	0.00**			
		Isoleucine	2.6	-0.12	0.14	-0.88	0.38			
		Proline	2.4	-0.03	0.07	-0.43	0.67			
		Galactonate	1.6	0.05	0.06	0.84	0.40			
Adjusted[a]	GC-MS (P)	(Int)	NA	(5.91)	(0.05)	(120.92)	(0.00***)	0.25	0.06	128.4
		C15	1.4	0.19	0.10	1.99	0.05*			
		Lactose	1.4	0.19	0.06	3.20	0.00**			
		BMI	1.1	-0.10	0.08	-1.27	0.21			
		Galactonate	1.7	0.06	0.06	1.02	0.31			
		Glutamic acid	3.3	-0.15	0.10	-1.47	0.14			
		Isoleucine	2.7	-0.08	0.14	-0.58	0.56			
		Proline	3.4	0.04	0.08	0.46	0.65			
High-fat dairy										
Unadjusted[a]	GC-MS (P)	(Int)	NA	(4.12)	(0.06)	(68.33)	(0.00***)	0.50	0.25	52.0
		C15	1.6	0.29	0.16	1.76	0.08			
		Glutamic acid	3.2	0.16	0.15	1.05	0.30			
		Proline	3.0	-0.21	0.12	-1.75	0.08			
		3-Hydroxyisobutyrate	2.4	-0.41	0.20	-2.04	0.04*			
Adjusted[a]	GC-MS (P)	(Int)	NA	(4.12)	(0.06)	(68.33)	(0.00***)	0.50	0.25	52.0
		C15	1.6	0.29	0.16	1.76	0.08			
		3-Hydroxyisobutyrate	2.4	-0.41	0.20	-2.04	0.04*			
		Glutamic acid	3.2	0.16	0.15	1.05	0.30			
		Proline	3.0	-0.21	0.12	-1.75	0.08			
Low-fat dairy										
Unadjusted[a]	GC-MS (P)	(Int)	NA	(5.67)	(0.04)	(125.96)	(0.00***)	0.27	0.07	131.4
		Galactonate	1.7	0.09	0.07	1.21	0.23			
		Lactose	1.4	0.21	0.07	3.21	0.00**			

Table S12. Multi-marker validation results for FIBs differentiating between fermented and non-fermented dairy intake by dairy group

	Analytical Platform (Biosample)	Biomarker	VIF	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
Total dairy										
		Glutamic acid	**3.0**	**-0.16**	**0.11**	**-1.40**	**0.16**			
		Isoleucine	**2.1**	**0.10**	**0.15**	**0.67**	**0.51**			
		Proline	**3.0**	**0.02**	**0.09**	**0.17**	**0.87**			
Adjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(5.71)**	**(0.06)**	**(97.98)**	**(0.00***)**	**0.31**	**0.10**	**130.0**
		Galactonate	**1.7**	**0.09**	**0.07**	**1.24**	**0.22**			
		Lactose	**1.4**	**0.22**	**0.07**	**3.29**	**0.00****			
		BMI	**1.1**	**-0.10**	**0.10**	**-1.09**	**0.28**			
		Glutamic acid	**3.3**	**-0.20**	**0.12**	**-1.66**	**0.10**			
		Isoleucine	**2.1**	**0.09**	**0.15**	**0.62**	**0.53**			
		Proline	**3.0**	**0.03**	**0.09**	**0.34**	**0.73**			
Total fermented dairy										
Unadjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(5.19)**	**(0.05)**	**(112.93)**	**(0.00***)**	**0.22**	**0.05**	**100.7**
		Galactonate	**1.6**	**0.07**	**0.07**	**0.95**	**0.34**			
		Glutamic acid	**2.4**	**-0.09**	**0.10**	**-0.87**	**0.38**			
		Lactose	**1.4**	**0.30**	**0.06**	**4.77**	**0.00*****			
		C15	**1.4**	**0.27**	**0.12**	**2.30**	**0.02***			
		Methionine	**3.0**	**-0.25**	**0.17**	**-1.48**	**0.14**			
		3-Hydroxyisobutyrate	**2.9**	**0.20**	**0.16**	**1.28**	**0.20**			
Adjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(5.27)**	**(0.05)**	**(98.90)**	**(0.00***)**	**0.20**	**0.04**	**100.7**
		C15	**1.5**	**0.22**	**0.12**	**1.83**	**0.07**			
		Lactose	**1.4**	**0.28**	**0.06**	**4.59**	**0.00*****			
		Age	**1.1**	**-0.26**	**0.10**	**-2.52**	**0.01***			
		Galactonate	**1.7**	**0.03**	**0.07**	**0.47**	**0.64**			
		Glutamic acid	**2.5**	**-0.12**	**0.10**	**-1.17**	**0.24**			
		Methionine	**3.1**	**-0.21**	**0.17**	**-1.22**	**0.23**			
		3-Hydroxyisobutyrate	**2.9**	**0.25**	**0.16**	**1.57**	**0.12**			
High-fat fermented dairy										
Unadjusted[a]	GC-MS (P)	(Int)	NA	(3.72)	(0.07)	(57.27)	(0.00***)	0.37	0.14	34.6
		C15	1.6	0.31	0.18	1.77	0.08			

Table S12. Multi-marker validation results for FIBs differentiating between fermented and non-fermented dairy intake by dairy group										
	Analytical Platform (Biosample)	**Biomarker**	**VIF**	**Coefficient**	**SE**	**t-value**	***p*-value**	**r_{ap}**	**R^2**	**MAE**
Total dairy										
		3-Hydroxyisobutyrate	2.4	-0.41	0.22	-1.87	0.06			
		Glutamic acid	3.1	0.22	0.17	1.31	0.19			
		Proline	3.0	-0.14	0.13	-1.07	0.29			
Adjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(3.80)**	**(0.08)**	**(46.43)**	**(0.00***)**	**0.40**	**0.16**	**35.1**
		C15	**1.3**	**0.34**	**0.16**	**2.18**	**0.03***			
		3-Hydroxyisobutyrate	**1.3**	**-0.40**	**0.16**	**-2.50**	**0.01***			
		BMI	**1.1**	**-0.19**	**0.14**	**-1.40**	**0.16**			
Low-fat fermented dairy										
Unadjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(4.91)**	**(0.06)**	**(80.62)**	**(0.00***)**	**0.25**	**0.06**	**93.1**
		C15	**1.4**	**0.26**	**0.15**	**1.65**	**0.10**			
		Lactose	**1.4**	**0.34**	**0.08**	**4.53**	**0.00*****			
		3-Hydroxyisobutyrate	**2.8**	**0.37**	**0.21**	**1.79**	**0.08**			
		Galactonate	**1.6**	**0.11**	**0.09**	**1.21**	**0.23**			
		Glutamic acid	**2.4**	**-0.15**	**0.13**	**-1.17**	**0.25**			
		Methionine	**3.0**	**-0.27**	**0.22**	**-1.23**	**0.22**			
Adjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(5.03)**	**(0.07)**	**(72.85)**	**(0.00***)**	**0.21**	**0.04**	**94.9**
		C15	**1.5**	**0.17**	**0.15**	**1.07**	**0.29**			
		Lactose	**1.4**	**0.32**	**0.07**	**4.31**	**0.00*****			
		3-Hydroxyisobutyrate	**3.3**	**0.44**	**0.22**	**2.05**	**0.04***			
		Age	**1.1**	**-0.40**	**0.14**	**-2.83**	**0.01****			
		Galactonate	**1.7**	**0.07**	**0.09**	**0.69**	**0.49**			
		Glutamic acid	**2.4**	**-0.19**	**0.13**	**-1.53**	**0.13**			
		Leucine	**2.4**	**-0.04**	**0.24**	**-0.16**	**0.87**			
		Methionine	**3.5**	**-0.19**	**0.23**	**-0.82**	**0.41**			
Total non-fermented dairy										
Unadjusted[a]	GC-MS (P)	(Int)	NA	(5.14)	(0.06)	(85.34)	(0.00***)	0.00	0.00	89.0
		3-Hydroxyisobutyrate	1.5	-0.25	0.16	-1.61	0.11			
		Galactonate	1.5	0.10	0.10	1.03	0.30			
		Proline	1.7	-0.01	0.10	-0.13	0.90			

Table S12. Multi-marker validation results for FIBs differentiating between fermented and non-fermented dairy intake by dairy group

	Analytical Platform (Biosample)	Biomarker	VIF	Coefficient	SE	t-value	*p*-value	r_{ap}	R^2	MAE
Total dairy										
Adjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(5.44)**	**(0.11)**	**(47.47)**	**(0.00***)**	**0.02**	**0.00**	**92.8**
		Valine_2TMS	**2.9**	**0.30**	**0.24**	**1.25**	**0.21**			
		3-Hydroxyisobutyrate	**2.9**	**-0.62**	**0.21**	**-2.95**	**0.00****			
		BMI	**1.1**	**-0.22**	**0.12**	**-1.77**	**0.08**			
		Sex	**1.2**	**-0.35**	**0.13**	**-2.74**	**0.01****			
		3-Phenyllactic acid	**2.1**	**0.33**	**0.18**	**1.82**	**0.07**			
High-fat non-fermented dairy										
Unadjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(3.64)**	**(0.06)**	**(61.75)**	**(0.00***)**	**0.40**	**0.16**	**27.4**
		C17	**1.4**	**0.38**	**0.17**	**2.30**	**0.02***			
		Isoleucine	**4.4**	**-0.98**	**0.28**	**-3.47**	**0.00*****			
		Leucine	**3.6**	**0.65**	**0.33**	**1.99**	**0.05***			
Adjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(3.64)**	**(0.06)**	**(61.75)**	**(0.00***)**	**0.40**	**0.16**	**27.4**
		C17	**1.4**	**0.38**	**0.17**	**2.30**	**0.02***			
		Isoleucine	**4.4**	**-0.98**	**0.28**	**-3.47**	**0.00*****			
		Leucine	**3.6**	**0.65**	**0.33**	**1.99**	**0.05***			
Low-fat non-fermented dairy										
Unadjusted[a]	GC-MS (P)	(Int)	NA	(5.01)	(0.07)	(73.72)	(0.00***)	-0.03	0.00	99.5
		3-Hydroxyisobutyrate	1.5	-0.27	0.18	-1.50	0.14			
		Galactonate	1.5	0.12	0.11	1.07	0.29			
		Proline	1.7	0.04	0.11	0.41	0.68			
Adjusted[a]	GC-MS (P)	**(Int)**	**NA**	**(5.33)**	**(0.13)**	**(41.72)**	**(0.00***)**	**0.11**	**0.01**	**96.1**
		Valine	**3.0**	**0.44**	**0.27**	**1.64**	**0.10**			
		3-Hydroxyisobutyrate	**2.9**	**-0.69**	**0.23**	**-2.95**	**0.00****			
		Sex	**1.2**	**-0.37**	**0.14**	**-2.63**	**0.01****			
		BMI	**1.1**	**-0.24**	**0.14**	**-1.75**	**0.08**			
		3-Phenyllactic acid	**2.1**	**0.37**	**0.20**	**1.82**	**0.07**			

BMI, body mass index; C15, pentadecanoic acid; C17, heptadecanoic acid; FIB, food intake biomarker; GC-MS, gas chromatography mass spectrometry; MAE, mean absolute error; NA, not applicable; P, plasma; aAIC, quasi-Akaike Information Criterion; SE, standard error; VIF, variance inflation factor. Significant results are bolded: *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

[a] Intercept (Int) values for the models are provided in brackets.

Figure S1. Differences in metabolite levels by sex-specific quintiles of milk intake: (a) plasma phenylalanine, (b) urinary lactose, (c) plasma Lewis A trisaccharide, and (d) urinary galactitol. Significance denoted by different letters ($p \leq 0.05$).

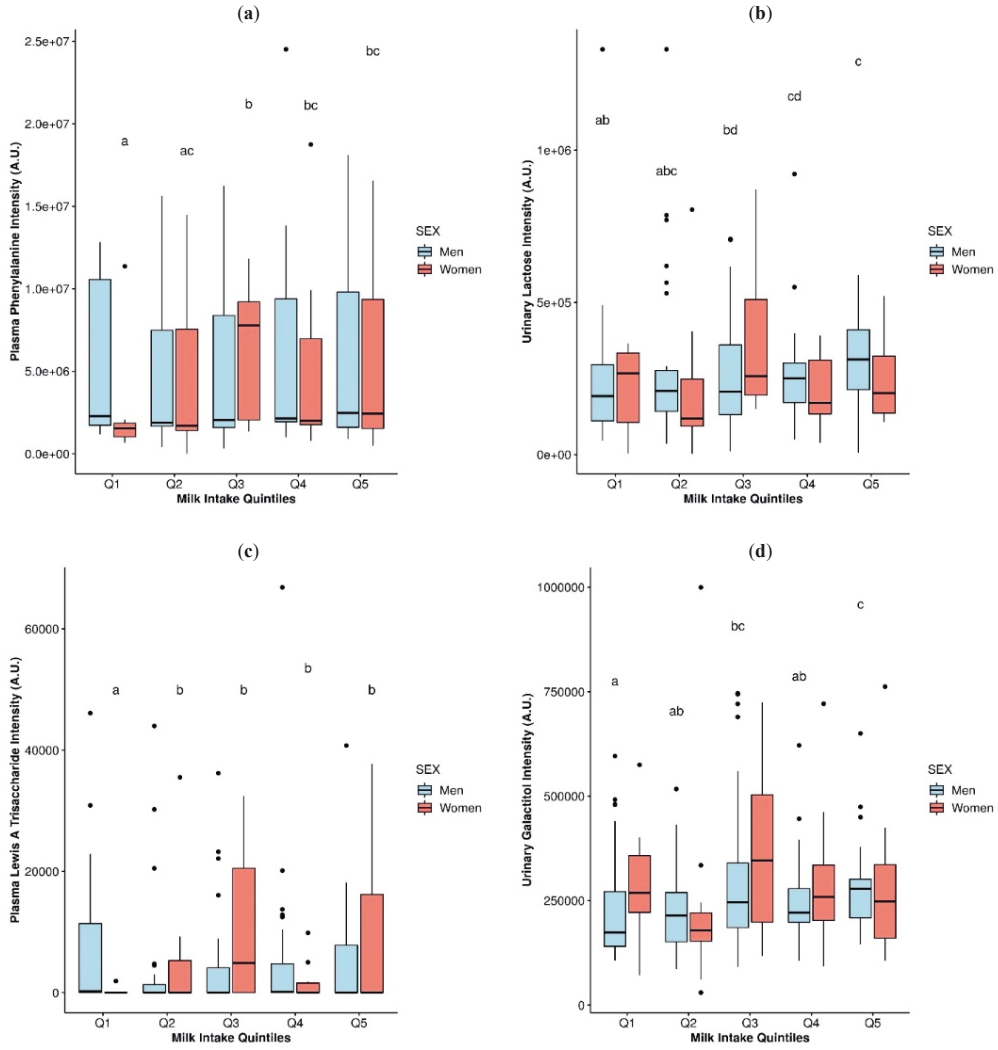


Figure S2. Levels of lactose metabolites. (a) plasma lactose (b) urinary lactose, (c) plasma galactose, (d) urinary galactose, (e) plasma galactonate, (f) urinary galactonate, (g) plasma galactitol, (h) urinary galactitol. Galactono-1,5-lactone was not detected in plasma or urine. LNP, lactase non-persistent; LP, lactase persistent; NA, genotype data not available.

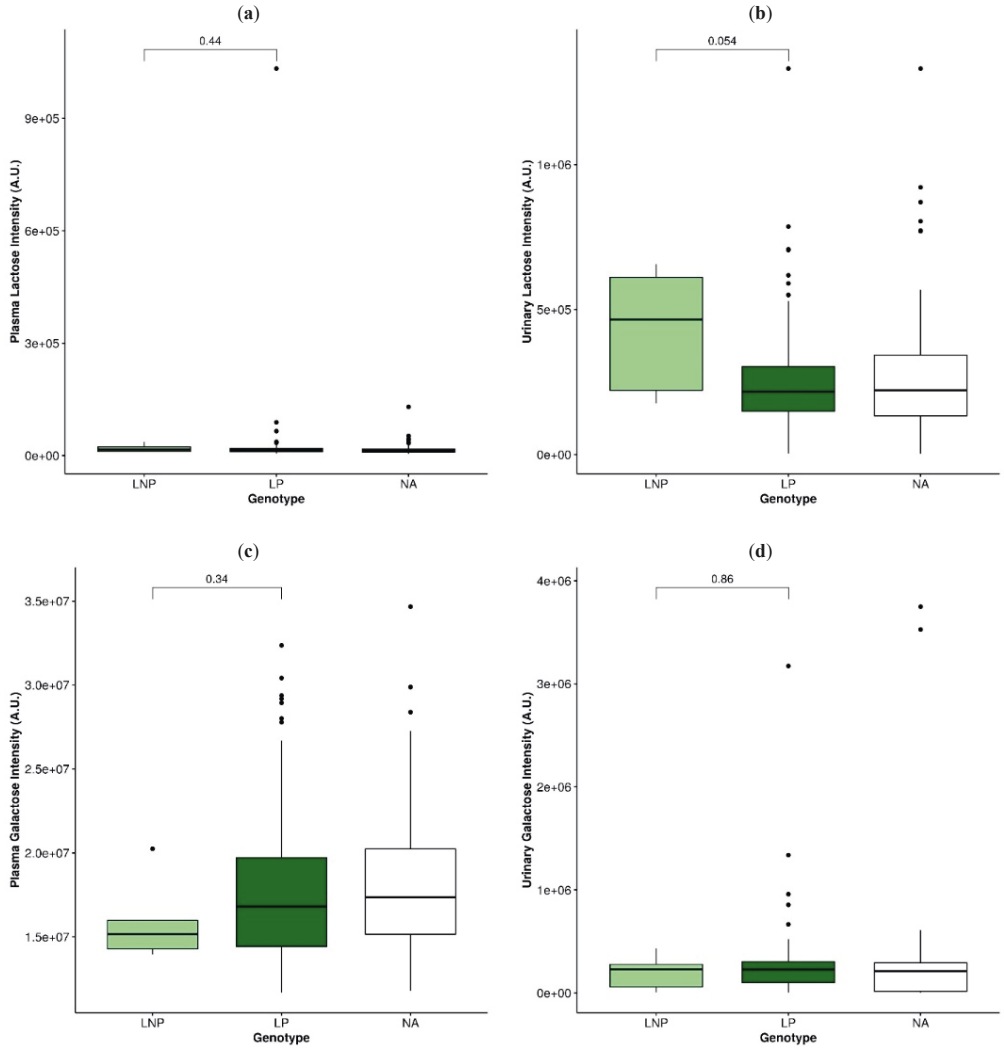


Figure S2 (cont'd)

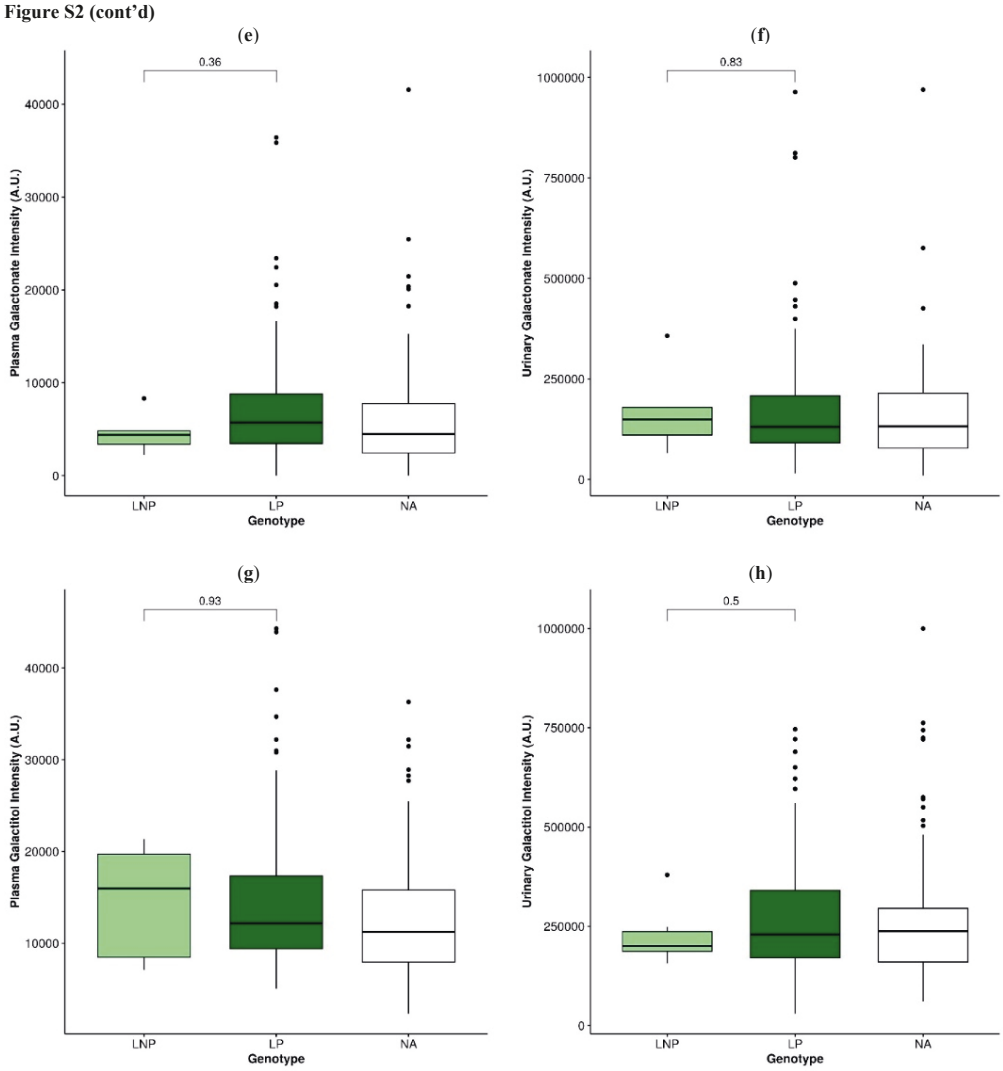


Figure S3. Levels of Lewis system-related oligosaccharides by secretion status. (a) plasma Lewis A trisaccharide, (b) plasma Blood Group H disaccharide, (c) urinary Blood Group H disaccharide. NA, genotype data not available.

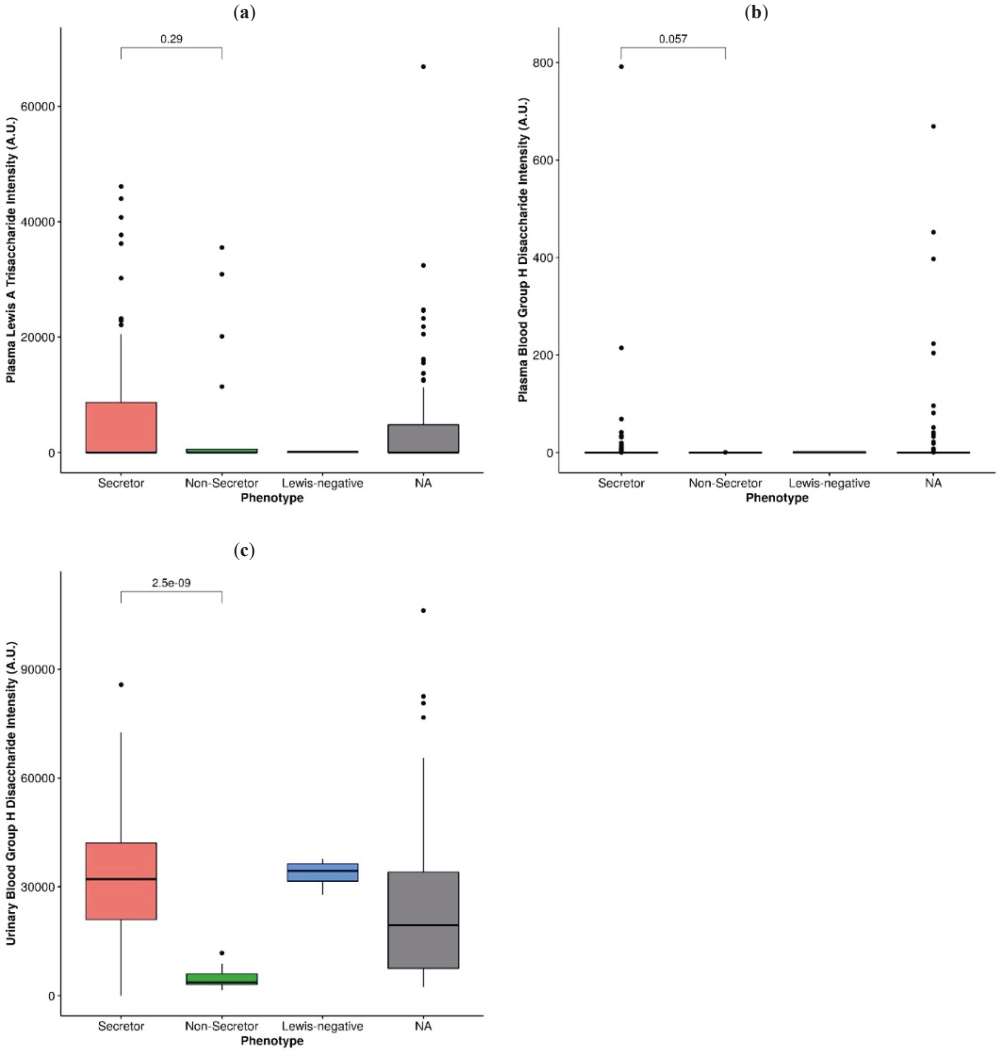


Figure S4. Differences in metabolite levels by sex-specific quintiles of cheese intake: (a) urinary indole-3-lactic acid, (b) plasma phenylalanyl-proline, (c) plasma proline. Significance denoted by different letters ($p \leq 0.05$).

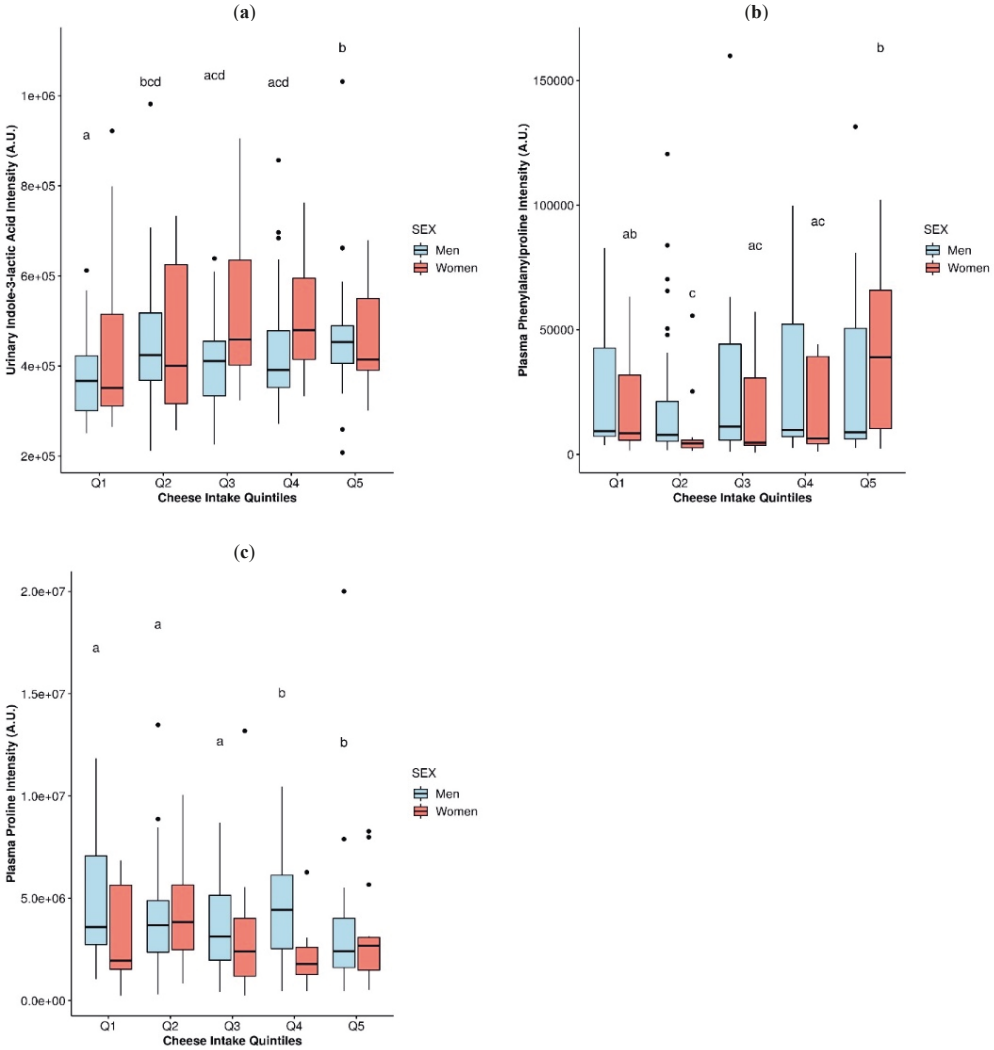


Figure S5. Differences in plasma levels of tyrosine by sex-specific quintiles of yogurt intake. Significance denoted by different letters ($p \leq 0.05$).

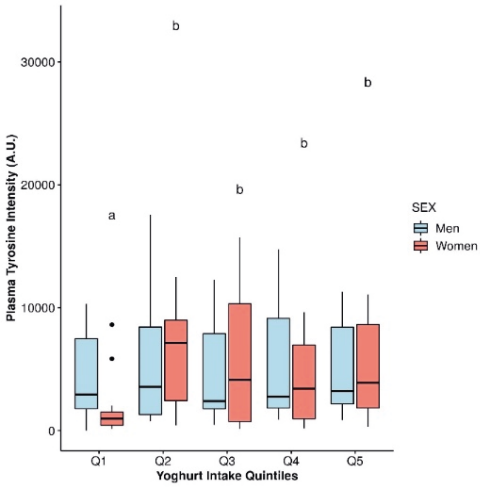


Figure S6. Differences in plasma levels of heptadecanoic acid by quintiles of total non-fermented dairy intake. Significance denoted by different letters ($p \leq 0.05$).

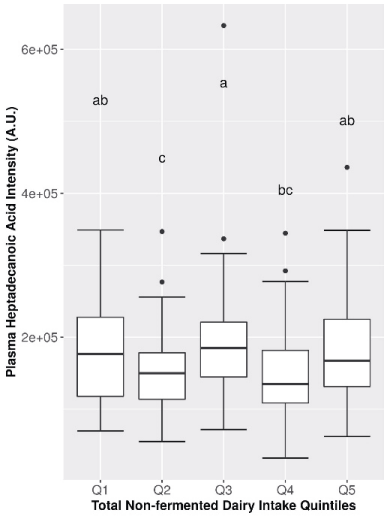
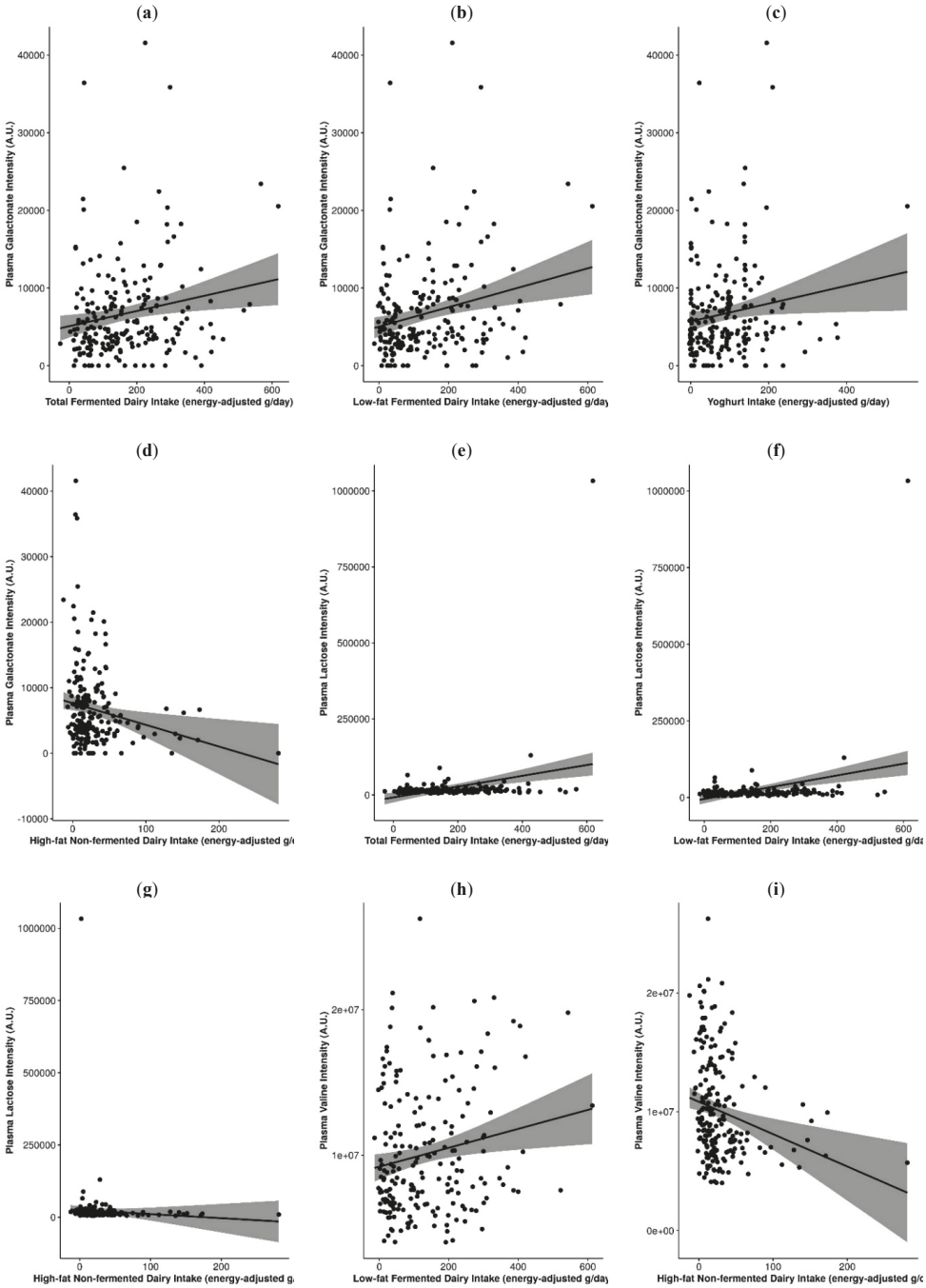


Figure S7. Plasma FIBs positively correlated with fermented dairy intake and negatively correlated with non-fermented dairy intake. Plasma galactonate significantly increasing with (a) total fermented dairy, (b) low-fat fermented dairy, and (c) yoghurt intake, and decreasing with (d) high-fat non-fermented dairy intake. Plasma lactose significantly increasing with (e) total fermented dairy and (f) low-fat fermented dairy intake, and decreasing with (g) high-fat non-fermented dairy intake. Plasma valine significantly increasing with (h) low-fat fermented dairy intake, and decreasing with (i) high-fat non-fermented dairy intake.



CHAPTER 5

Associations between dairy fat intake, milk-derived free fatty acids, and cardiometabolic risk in Dutch adults

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Submitted

Abstract

Purpose: Milk-derived free fatty acids (FFAs) may act as both biomarkers of intake and metabolic effect. In this study we explored associations between different types of dairy consumption, a selection of milk-derived free fatty acids, and cardiometabolic disease (CMD) risk factors.

Methods: Sixty-seven FFAs were quantified in the plasma of 131 free-living Dutch adults (median 60 years) using gas chromatography-flame ionization detector. Intakes of different dairy foods and groups were assessed using a food frequency questionnaire. Twelve different CMD risk factors were analyzed. Multiple linear regressions were used to evaluate the associations under study.

Results: Among the fully-adjusted models, 5 long-chain unsaturated FFAs (C18.1 t13+c6+c7+u, C18:2 c9t11+u, C20:1 c11, C20:3 c8c11c14, and C20:4 c5c8c11c14), 2 medium-chain saturated FFAs (C15, C15 *iso*), and a *trans* FFA (C16:1 t9) were positively associated with at least one variable of dairy intake, as well as plasma total and LDL cholesterol, blood pressure, and SCORE. A long-chain PUFA associated with high-fat fermented dairy intake (C18:2 t9t12), was negatively associated with serum triglyceride levels, and a long-chain saturated FFA associated with cheese intake (C18:1 u1) was negatively associated with plasma LDL cholesterol and serum triglyceride levels. No clear associations were observed between dairy intake and CMD risk factors.

Conclusion: Milk-derived FFAs could act as sensitive biomarkers for dairy intake and metabolism, allowing the association between dairy and CMD risk to be more precisely evaluated.

Introduction

Cardiometabolic diseases (CMDs), encompassing cardiovascular disease and type II diabetes, represent one of the largest health and socioeconomic burdens to modern society. In Europe, cardiovascular disease morbidity affects more than 85 million people, leading to ~4 million annual deaths (1). Proper nutrition is considered the primary lifestyle approach for preventing and managing CMD risk. Diets abundant in fruits, vegetables, whole grains, nuts, and legumes contribute to lowering CMD risk, while consumption of processed meats, refined grains, and sugar-sweetened beverages are considered detrimental (2). Particular nutrients in foods have also been adversely associated with CMD risk, including sugar, sodium, *trans* (unsaturated) fat, and saturated fat (2).

Dairy products have had a contested role in the dietary management of CMDs due to suggested beneficial as well as adverse health effects. While dairy foods are rich in macro- and micronutrients considered important for growth and development, they can also have a high saturated fat content (contributing 25-30% of all saturated fat intake in the European diet) (3). Earlier studies conducted in the 1960-70s have reported that saturated fat adversely affects low-density lipoprotein cholesterol (LDL-C) levels in blood, which in turn increases CMD pathogenesis and progression (3, 4). In view of this, many subsequent observational studies have compared high- and low-fat dairy intake on CMD risk, but the findings have been equivocal. A meta-analysis of prospective cohort studies revealed that total and low-fat dairy intake (but not high-fat dairy intake) was associated with a lower risk of hypertension (5), while several studies have revealed the merits of consuming full-fat dairy products on reducing central adiposity risk (6, 7) and increasing serum high-density lipoprotein cholesterol (HDL-C) (8). In a recent systematic review of randomized controlled trials, Duarte *et al.* (9) reported that the consumption of dairy products (as a source of saturated fat) may improve some CMD risk factors compared with consumption of other animal sources of saturated fat. This finding lends support to the importance of considering the whole dairy matrix on disease outcomes. An increasing body of evidence also supports the differential role of distinct dairy foods with unique nutritional and/or microbial profiles on CMD risk (10). For instance, consumption of yoghurt, a fermented dairy food, has been consistently associated with a reduced risk of type II diabetes (11) and combined cardiovascular disease (12).

A limitation of the above studies (which could partly explain the equivocal findings) is the reliance on subjective, self-reported dietary assessment methods. Here, food intake biomarkers (FIBs) could act as a more objective and accurate strategy for estimating dairy intake, and can lead to more consistent findings for associations between dairy intake and CMD risk factors (13). Several fatty acids have been proposed as FIBs for dairy fat intake, including pentadecanoic acid (C15), heptadecanoic acid (C17), and *trans*-palmitoleic acid (C16:1 t9) (14-20), but these FIBs have not been thoroughly validated, and/or cannot discriminate the intake of specific dairy foods. Additionally, milk fat is a highly complex mixture of several thousand species of lipids, including ~400 fatty acids, which have distinct physiological importance and nutritive potential (21, 22). Longer chain saturated fatty acids such as (*e.g.*, C14, C16) as well as certain *trans* fatty acids are known to positively associate with total and LDL-C (23) and type II diabetes risk (24), while saturated fatty acids with shorter chain length (C6-C10), monounsaturated, and polyunsaturated fatty acids (MUFA and PUFA) are generally regarded as cardio-protective (25-27). Surprisingly little is known about the relationships between milk-derived fatty acids with dairy intake, or how they associate with CMD risk. Recently, Drouin-Cartier *et al.* (25) evaluated plasma metabolite profiles associated with total dairy intake and risk of type II diabetes, and found 38 metabolites associated with dairy intake and lower type II diabetes risk. However, such comprehensive studies are fairly scarce and there remains a need to consider the interplay between different dairy foods, lipids and metabolites, and CMD risk factors.

The objective of the current study is therefore to evaluate associations between dairy intake, milk-derived free fatty acids (FFAs), and CMD risk parameters in a free-living adult population. To achieve a

comprehensive analysis, we targeted 67 FFAs with high abundance in milk and looked at their associations with different dairy intake groups (dairy fat, fermented and non-fermented dairy, and specific dairy foods), and a wide range of CMD risk factors and composite risk scores.

Methods

Study design and population

The Nutrition Questionnaires plus (NQplus) study is a prospective cohort study comprising 2048 Dutch adults (20 to 70 years) living in or around Wageningen, The Netherlands. NQplus was initiated as an 'add-on' study to the National Dietary Assessment Reference Database (NDARD) project, and full details of NQplus and NDARD have been published previously (28, 29). Participants were recruited and enrolled in the study between June 2011 and February 2013. Extensive data were collected at baseline, including an assessment of habitual dietary intake by food frequency questionnaire (FFQ) and/or 24-h recall, background demographics, health, anthropometric, and lifestyle data. Fasting blood samples and 24-h urine samples were also collected. The study was approved by the ethical committee of Wageningen University & Research and conducted in agreement with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the start of the study.

For the present study, we selected n = 131 NQplus participants for targeted FFA analysis from n = 228 who had a plasma sample collected within ±14 days of completing an FFQ (30). The selection of this smaller group was necessary to attain a number of samples that could be reasonably measured within time and economic restraints. To ensure a balance of participants across all dairy groups/foods assessed, ~20 participants were randomly selected from the low (Q1), mid (Q3), and high (Q5) quintiles of intake of each dairy group/food assessed.

Food frequency questionnaire and levels of dairy consumption

A full description of the semi-quantitative FFQ used to assess habitual dietary intake has been described in the study design papers for NQplus and NDARD (28, 29). The semi-quantitative FFQ was self-administered and completed online, with ten frequency categories ranging from 'never' to '6–7 days per week'. Portion sizes were estimated using commonly used household measures. Total food or nutrient intakes (in g/day) were determined by multiplying consumption frequency by portion size and nutrient content as defined in the Dutch food composition tables (31). Out of 216 total food items in the FFQ, 39 were identified as dairy products, which were further classified into dairy subgroups (**Table S1**). This FFQ has been previously validated for various dairy foods and food groups, including milk, yogurt, cheese, total fermented dairy, and total non-fermented dairy (against multiple 24-h recalls) (32), which are used in the current study for evaluation of the respective candidate FIBs. Total dairy, fermented dairy, and non-fermented dairy groups were further stratified into high-fat groups, which included all full-fat dairy products, and low-fat groups, which included semi-skim and skim dairy products. Fat content (g/100g) for all dairy products was determined based on the values reported in the Dutch Food Composition Table (31) and classifications of products as skim, semi-skim, and full-fat were based on the guidelines set by the Dutch Dairy Commodities Act (see **Table S1**).

Cardiometabolic disease risk parameters

A full description of the CMD risk parameters collected for NQplus has been described previously (29). Height and weight were determined using a stadiometer (SECA, Germany, nearest 0.1 cm) and a digital weighing scale (SECA, nearest 0.1 kg), respectively. BMI was calculated by dividing weight (in kg) by height (in m^2). Waist circumference was determined using a non-flexible measuring tape (SECA 201, nearest 0.5 cm); measurements were taken twice and averaged. Systolic and diastolic blood pressure (SBP and DBP) was measured using a digital blood pressure monitor (IntelliSense HEM-907, Omron Healthcare, USA); the first

measurement was omitted and the second to up to the sixth measurement were averaged. Fasting plasma glucose, total, HDL-C, and serum triglycerides were analysed using Dimension Vista 1500 automated analyser (Siemens, Erlangen, Germany) or Roche Modular P800 chemistry analyser (Roche Diagnostics, Indianapolis, USA). Plasma LDL-C was calculated with the Friedewald equation (33). Blood haemoglobin A1c (HbA1c) was determined with HPLC (ADAMS A1c HA-8160 analyser, A. Menarini Diagnostics). Participants were characterized as having hypertension, suboptimal cholesterol, or type II diabetes based on the cut-offs and definitions described in relevant guidelines of the European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) (34-36), and having metabolic syndrome based on the harmonized guidelines of the International Diabetes Federation (IDF) *et al.* (37).

Two further composite risk scores were included in our analyses. Firstly, a continuous metabolic syndrome (MetS) score was constructed based on summed age- and sex-adjusted standardized residuals (z-scores) of all individual MetS parameters that incorporates plasma glucose, SBP, DBP, HDL-C, serum triglycerides, waist circumference as risk factors (38-40). Since HDL-C is inversely associated with CMD risk, residuals for this parameter were multiplied by -1 prior to summing. Secondly, the 10-year risk of fatal cardiovascular disease was evaluated using the European Systematic COronary Risk Evaluation (SCORE) low-risk country chart (41, 42), which incorporates sex, age, smoking status, SBP, and total cholesterol as risk factors. For the calculation of SCORE, a 'smoker' was considered to encompass current smokers and former smokers who quit >35 years old, and 'non-smoker' as never smokers and former smokers who quit <35 years old, as this age cut-off has been previously documented as a critical smoking cessation age to prolong life expectancy (43).

Covariates

Data on education level, smoking status, physical activity, and alcohol consumption were collected via standardized questionnaires (29). Participants were classified as having 'low', 'intermediate', or 'high' education based on their highest levels of completed education (no education or primary/lower vocational education, lower secondary or intermediate vocational education, or higher secondary/vocational education or university). The criteria used to define 'smoker' and 'non-smoker' are outlined above. Information on the participants' usual physical activity (min/week spent on sedentary, light, moderate and vigorous intensity activities) over the past four weeks was obtained using the validated Activity Questionnaire for Adults and Adolescents (AQuAA) (44). Intake levels of alcohol and different foods were assessed by FFQ. Covariate selection was based on the current scientific literature and statistical testing (as described in the statistical analysis).

Measurement of free fatty acid concentrations in plasma

Ethylenediaminetetraacetic acid (EDTA) plasma collected for NQplus was stored in the biobank at -80°C. The targeted panel comprised 67 FFAs previously detected with the highest abundance in the milk fat of Swiss cows (45). In addition, several FFA groups were determined based on the sums of individual FFAs (**Table S2**). Immediately prior to analysis, plasma samples were thawed on ice and were prepared for analysis by adding 15 µL of the internal standard (C13, 7 µg/15 µL) to 100 µL of plasma, followed by methylation of FFAs with MeOH/HCl at 25°C for 45 min using methods described previously (46, 47). A post-reaction treatment for neutralization was performed with 350 µL Na_2CO_3, and extraction was performed with 300 µL hexane. The FFA concentrations in plasma were determined using 0.5 µL injection of the hexane solution and analyzed using an Agilent 6890 high-resolution gas chromatograph equipped with a capillary column (100-m CP-Sil 88, Varian BV, Middleburg, Netherlands) and a flame ionization detector.

Statistical analysis

Participant characteristics are reported as mean (standard deviation) for normally distributed variables, or medians (interquartile range) for skewed variables. To permit comparability with a previous study where we assessed correlations between plasma C15 and C17 with various dairy groups (30), Spearman's correlation coefficients (r_s) were calculated as an initial step to assess the strength of the associations between dairy intakes (energy-adjusted g/day) and FFA concentrations (mg/L plasma); *p*-values are presented as raw and false discovery rate (FDR)-adjusted using the method of Benjamini and Hochberg (48). Correlation coefficients of ≥0.50 were considered good, 0.20-0.49 as moderate, and <0.20 as poor (39).

Multivariable adjusted linear regression and restricted cubic spline regression were used to evaluate the associations between self-reported dairy intakes, FFAs, and CMD risk factors. The assumption of linear relationships between exposure and outcome variables were tested using likelihood ratio tests of model deviance and Wald tests of spline coefficients. If tests were statistically significant, associations were visually inspected to confirm the presence of a true non-linear relationship and not due to artificial curves driven by outliers. All tests for non-linearity revealed that the dose-response association of dairy intake with FFAs and CMD risk parameters, and between FFAs and CMD risk parameters, could be considered linear. Thus, only linear regressions are presented in the results. Prior to association analyses, intakes of dairy foods were energy-adjusted using the commonly used residual method (49). FFAs that were not detected in at least a third of participants were removed to select the most suitable candidate FIBs of dairy intake. CMD risk parameters acting as dependent variables that were not normally distributed (BMI, HbA1c, plasma glucose, serum triglycerides, and SCORE) were log transformed. Additionally, all variables were normalized by z-scores prior to analysis to allow comparability across associations. Analyses were performed unadjusted (Model 0), adjusted for age and sex (Model 1) + physical activity, smoking, and education level (Model 2) + dietary factors (Model 3). For associations with continuous MetS as a dependent variable, which already takes into account age and sex, analyses were performed unadjusted (Model 0) and fully-adjusted for smoking, physical activity, education, and dietary factors (Model 3). For associations with SCORE as a dependent variable, which already takes into account age, sex, and smoking status, analyses were performed unadjusted (Model 0) and fully-adjusted for physical activity, education, and dietary factors (Model 3). Dietary factors included in the fully-adjusted models were dependent on the association studied, and included fish (dairy intake and FFAs), or alcohol, vegetables, fruits, and meat (dairy intake and CMD risk factors, FFAs and CMD risk factors). No other foods or food groups (listed in **Table 1**) were found to be strongly correlated with dairy intake, FFAs, or with CMD risk factors, and were thus not included in the models.

Further, we intended to examine potential mediation of the association between dairy intake and CMD risk factors by milk-derived FFAs by independently adding the FFAs to fully adjusted regression models. However, since there were no clear associations between dairy intake and CMD risk factors, we did not further examine the role of the FFAs as potential mediators.

All analyses were performed in R (Version 3.6.3) (50). Visualizations of the intercorrelations between FFAs was performed using the corrplot R package (51), and visualizations of the associations were performed using the ggplot2 R package (52). The script for the circular plots were adapted from Ladroue (53). For all models, the level of significance was set at $p \leq 0.05$. However, due to the large number of associations examined in this work, the models were also adjusted for multiple comparisons (48). Both significant raw and FDR-adjusted *p*-values are relevant and presented; significant FDR-adjusted results are highlighted where appropriate to help focus the findings.

Results

Population characteristics

The characteristics of the study population are presented in **Table 1**. The median age of the participants was 60 years, and a majority were highly educated (55%) and non-smokers (69%). About half (54%) of the participants were overweight or obese, 33% had hypertension, 76% had suboptimal cholesterol levels, and 2% had diabetes. The distribution of the continuous MetS score and SCORE is presented in **Figure S1**. Significant differences observed between men and women for several CMD risk parameters (waist circumference, blood pressure, HDL-C, plasma glucose) reflected the patterns observed in the total NQplus population (29).

The median total dairy intake of the study participants was 306 g/day, with dairy fat accounting for approximately 15 g/day (5%) of average daily energy intake. Low-fat dairy products (comprising mostly low-fat fermented dairy) were consumed at a higher level than high-fat dairy products (median: 237 *vs.* 40 g/day), and fermented dairy products had a higher level of intake compared to non-fermented dairy products (median: 151 *vs.* 88 g/day). No significant differences in dairy intakes were observed between men and women. Among other dietary factors, men had significantly higher intake of total energy and several nutrients (fat, carbohydrates, fibre, protein, sodium) and foods (alcohol, potatoes, meat, sauces, and snack foods) compared to women. Significant differences were also observed for coffee intake between sexes (due to the ranking of the data by the Wilcoxon test), but medians were comparable.

Mean plasma FFA concentrations are presented in **Table S3**. Seventeen of the original 67 FFAs were not detected in at least a third of participants and thus removed from the analyses (primarily short-chain FFAs that are likely to be metabolized rapidly). Out of the remaining 50 FFAs, the majority were detected in plasma at concentrations of less than 1 mg/L, while five FFAs (C16, C18:1 c9, C18:2 c9c12, C18, and C20:4 c5c8c11c14) were detected at much higher concentrations compared to all other FFAs (12.6 to 75.3 mg/L). An inter-correlation analysis revealed that a large number of FFAs were significantly positively correlated with each other (**Figure S2**).

Table 1. Characteristics of the study population (*n* = 131) [a]

Characteristic	All (*n* = 131)	Men (*n* = 86)	Women (*n* = 45)	*p*-value
Demographics				
Age, years	60 (48 - 65)	61 (49 - 66)	58 (45 - 63)	0.11
Education, n (%)				0.38
Low	11 (8)	8 (9)	3 (7)	
Intermediate	48 (37)	29 (34)	19 (43)	
High	71 (55)	49 (57)	22 (50)	
Smoking status, n (%)				0.33
Smoker	37 (30.8)	27 (33.8)	10 (25.0)	
Non-smoker	83 (69.2)	53 (66.3)	30 (75.0)	
Physical activity, min/week	1922 (1070 - 2748)	1911 (934 - 2725)	1930 (1215 - 2760)	0.44
Supplement use, n (%)	59 (45)	38 (44)	21 (47)	0.48
Dietary Factors				
Total energy intake, kcal/day	2207 ± 507	2355 ± 477	1924 ± 443	**<0.001*****
Macronutrients, g/day (% energy)				
Carbohydrates	233 ± 60 (42)	246 ± 59 (42)	208 ± 54 (43)	**<0.001*****
Protein	79 ± 17 (14)	83 ± 17 (14)	71 ± 14 (15)	**<0.001*****
Fat	89 ± 27 (36)	95 ± 26 (36)	79 ± 25 (37)	**<0.001*****
Fibre, g/day	25 ± 8	26 ± 8	23 ± 6	**0.01****
Sodium, mg/day	2357 ± 676	2524 ± 704	2040 ± 486	**<0.001*****
Dairy fat, g/day	15 (9 - 24)	15 (9 - 24)	17 (10 - 23)	0.75

Dairy foods and groups, g/day				
Total dairy	306 (162 - 400)	289 (162 - 382)	329 (164 - 470)	0.18
High-fat dairy	40 (14 - 85)	38 (16 - 89)	46 (14 - 82)	0.85
Low-fat dairy	237 (90 - 354)	228 (73 - 316)	255 (125 - 381)	0.75
Total FD	151 (60 - 255)	152 (73 - 250)	151 (48 - 260)	0.61
High-fat FD	18 (3 - 49)	17 (2 - 49)	25 (3 - 49)	0.38
Low-fat FD	106 (25 - 226)	112 (29 - 220)	85 (21 - 230)	0.17
Total NFD	88 (30 - 201)	86 (26 - 178)	88 (37 - 262)	0.17
High-fat NFD	14 (6 - 31)	17 (6 - 34)	11 (5 - 24)	0.86
Low-fat NFD	56 (0 - 152)	46 (0 - 139)	64 (12 - 211)	0.09
Milk	76 (13 - 189)	71 (13 - 153)	84 (16 - 236)	0.36
Cheese	30 (12 - 50)	28 (13 - 52)	31 (11 - 46)	0.93
Yoghurt	84 (11 - 139)	95 (13 - 139)	73 (7 - 139)	0.84
Other foods and groups, g/day				
Coffee	406 (261 - 638)	406 (406 - 638)	406 (174 - 609)	**0.002****
Tea	174 (65 - 406)	174 (27 - 406)	174 (67 - 406)	0.25
Alcoholic drinks	112 (13 - 289)	180 (60 - 386)	17 (0 - 112)	**<0.001*****
Soft drinks	13 (0 - 53)	18 (0 - 54)	4 (0 - 27)	0.08
Fruits	161 (79 - 235)	159 (76 - 234)	210 (81 - 237)	0.60
Vegetables	139 (94 - 193)	129 (91 - 169)	160 (98 - 208)	**0.02***
Bread	130 (96 - 166)	133 (101 - 169)	108 (76 - 139)	**0.01***
Other cereals and grains	47 (32 - 88)	49 (33 - 100)	45 (30 - 84)	0.38
Potatoes	67 (37 - 87)	67 (37 - 87)	37 (22 - 67)	**0.005****
Legumes	38 (19 - 75)	38 (22 - 78)	34 (17 - 73)	0.55
Meat products	74 (49 - 96)	84 (58 - 121)	66 (42 - 82)	**0.002****
Eggs and egg products	14 (7 - 18)	14 (7 - 18)	18 (7 - 18)	0.62
Fish	11 (6 - 16)	11 (7 - 16)	11 (4 - 16)	0.88
Nuts and seeds	15 (6 - 26)	14 (6 - 26)	15 (4 - 27)	0.91
Sauces, spreads and cooking fats	43 (29 - 57)	46 (33 - 59)	35 (17 - 47)	**0.001*****
Salty and processed snack foods	38 (19 - 65)	43 (25 - 74)	29 (13 - 50)	**0.01****
Sugary confectionary and desserts	78 (50 - 112)	79 (50 - 115)	68 (51 - 107)	0.54
Cardiometabolic factors				
BMI, kg/m^2	25.5 (23.2 – 27.1)	25.8 (23.4 – 28.2)	24.5 (22.7 – 26.5)	0.06
BMI category, n (%)				0.10
Underweight (<18.5 kg/m^2)	2 (2)	1 (1)	1 (2)	
Normal weight (18.5-24.9 kg/m^2)	58 (44)	32 (37)	26 (58)	
Overweight or obese (≥25-29.9 kg/m^2)	71 (54)	53 (62)	18 (40)	
Waist circumference, cm	91.7 ± 11.6	95.0 ± 10.3	85.4 ± 11.5	**<0.001*****
Diastolic blood pressure, mm Hg	74.0 ± 10.4	76.1 ± 9.7	70.0 ± 10.7	**0.002****
Systolic blood pressure, mm Hg	127.9 ± 16.8	131.7 ± 15.8	120.7 ± 16.4	**<0.001*****
Hypertension, n (%)				**0.03***
Hypertension[b]	33 (25)	26 (30)	7 (16)	
Normal or optimal	98 (75)	60 (70)	38 (84)	
Hypertension treatment, n (%)				0.23
Being treated (with medication and/or diet)	20 (15)	16 (19)	4 (9)	
Not being treated	111 (85)	70 (81)	41 (91)	
Plasma total cholesterol, mmol/L	5.3 ± 1.0	5.3 ± 1.0	5.4 ± 1.1	0.054
Plasma LDL cholesterol, mmol/L	3.3 ± 0.9	3.4 ± 0.9	3.2 ± 0.9	0.19
Plasma HDL cholesterol, mmol/L	1.5 ± 0.4	1.4 ± 0.3	1.8 ± 0.5	**<0.001*****
Serum triglycerides, mmol/L	1.0 (0.8 - 1.4)	1.1 (0.8 - 1.4)	1.0 (0.8 - 1.2)	0.15
Suboptimal cholesterol, n (%)	100 (76)	71 (83)	29 (64)	**0.04***

High cholesterol treatment, n (%)				0.14
Being treated (with medication and/or diet)	10 (8)	9 (11)	1 (2)	
Not being treated	121 (92)	77 (89)	44 (98)	
HbA1c, mmol/mol	36.0 (34.0 – 37.8)	35.5 (34.0 – 37.0)	36.0 (34.0 – 38.9)	0.47
Fasting plasma glucose, mmol/L	5.3 (5.0 – 5.6)	5.4 (5.1 – 5.7)	5.0 (4.8 – 5.5)	**<0.001*** **
Diabetes, n (%)	2 (2)	1 (1)	1 (2)	0.38
Diabetes treatment, n (%)				0.40
Being treated (with medication and/or diet)	2 (1)	1 (1)	1 (2)	
Not being treated	129 (99)	85 (99)	44 (98)	
Metabolic syndrome, n (%)	14 (10.7)	10 (11.6)	4 (8.9)	0.74
SCORE, n (%)	120	80	40	0.46
≥15 %	3 (2)	3 (4)	0 (0)	
10-14 %	8 (7)	8 (10)	0 (0)	
5-9 %	25 (21)	21 (26)	4 (10)	
1-4%	52 (43)	33 (41)	19 (47)	
<1 %	32 (27)	15 (19)	17 (43)	

BMI, body mass index; FD, fermented dairy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NFD, non-fermented dairy; SCORE, Systematic COronary Risk Evaluation. Significant results are bolded: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
[a] Values are presented as mean ± SD, or median (IQR). Missing values: education (*n* = 1), smoking status (*n* = 11), physical activity (minutes/week) (*n* = 20), LDL (*n* = 1), HDL (*n* = 1), Hb1Ac (*n* = 1), glucose (*n* = 1), SCORE (*n* = 11). Differences in characteristics between sexes were assessed using the t-test (for normally-distributed continuous variables), Wilcoxon test (for skewed continuous variables), or chi-squared test (for categorical variables).
[b] Inclusive of Grade 1 hypertension, Grade 2 hypertension, and isolated systolic hypertension.

Free fatty acids associated with dairy intake

Among the 50 FFAs listed in **Table S3**, 21 were positively correlated with one of the variables of dairy intake (raw $p \leq 0.05$) (**Table S4)**. After adjusting for multiple comparisons, moderate correlations for dairy intake and 5 FFAs remained significant, specifically for the intake of dairy fat (C15, C16:1 t9, C16 *iso*, C17 *iso*, and C18:2 c9t11+u; r_s = 0.26, 0.32, 0.30, 0.35, and 0.29, respectively) and cheese (C18:2 c9t11+u; r_s = 0.30) (all FDR $p \leq 0.05$). Out of the summed groups, summed conjugated linoleic acids (CLA) were also significantly positively correlated with dairy fat (r_s = 0.29) and cheese intake (r_s = 0.31), and summed C15 and C17, with and without C16:1 t9, was positively associated with total fermented dairy intake (r_s = 0.26) (FDR $p \leq 0.05$).

The correlations between FFAs and dairy intake were largely confirmed in the multiple linear regression models (**Table S5**). In the fully-adjusted model (**Figure 1**), positive associations were observed between 14 individual FFAs and multiple dairy groups/foods (raw $p \leq 0.05$; non-significant after FDR adjustment). The strongest associations were observed between the medium-chain unsaturated FFA C16:1 t9 with dairy fat (standardized β (Std. β) = 4.9, R^2 = 0.2), high-fat dairy (Std. β = 4.8, R^2 = 0.2), high-fat fermented dairy (Std. β = 4.9, R^2 = 0.2), and cheese (Std. β = 3.5, R^2 = 0.1) (raw $p \leq 0.05$). Significant associations between C15 with dairy fat (Std. β = 1.0, R^2 = 0.1) and high-fat dairy (Std. β = 1.0, R^2 = 0.2) intake were also observed albeit with lower effect size (no significant associations were observed for C17).

Additionally, the long-chain FFAs C17 *iso*, C22, and C20:3 c8c11c14 were found to be positively associated with the intake of multiple dairy groups/foods; the latter two FFAs were primarily associated with low-fat dairy foods/groups (raw $p \leq 0.05$). Among the summed FFA groups, sum of CLA was positively associated with dairy fat intake, while sum of C18:2t with CLA was positively associated with cheese intake. A small number of negative associations were observed between FFAs and primarily low-fat and non-fermented dairy groups (including milk), namely the long-chain unsaturated FFAs C20:1 c11, C17:1 c10, C18:2 t9t12, C18:3 c6c9c12, C18:1 c9, the medium-chain unsaturated FFA C16:1 u1, in addition to sum of C18:2t without CLA and sum of MUFA.

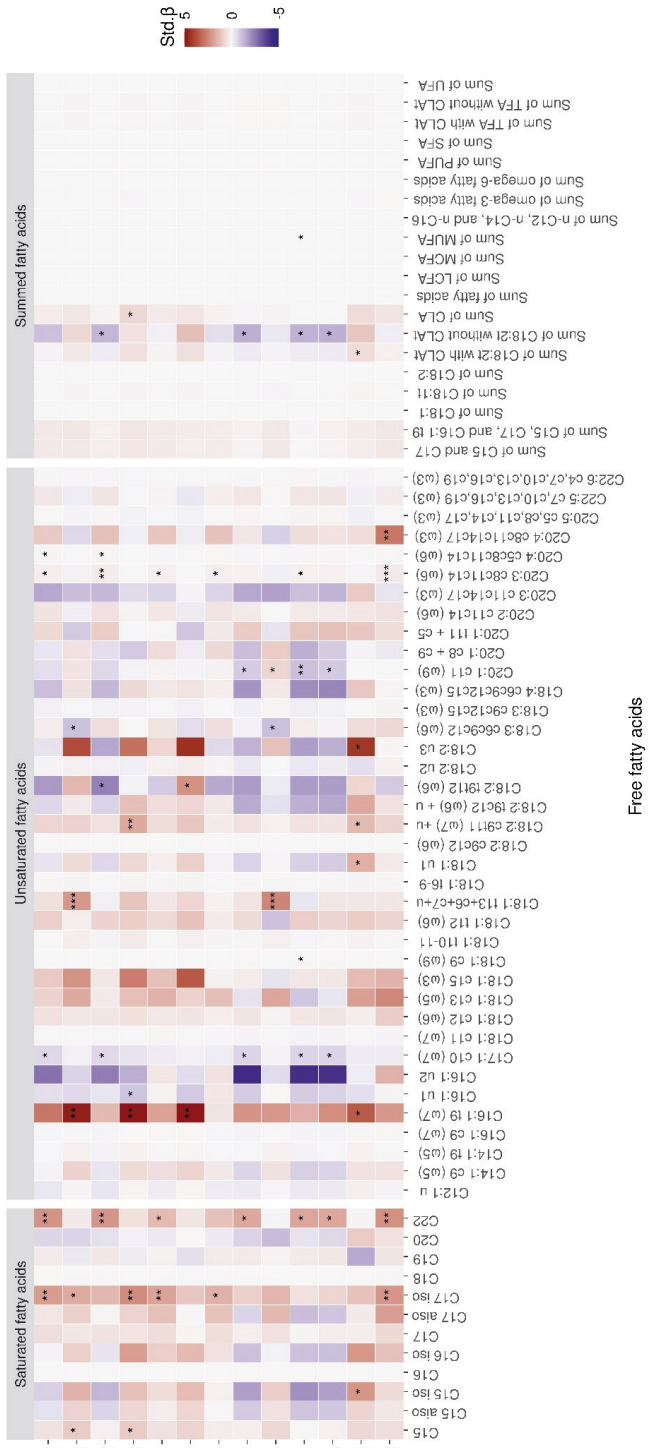

Figure 1. Summary of the associations between FFAs and dairy intake groups in the fully-adjusted model (Model 3). Individual and summed FFAs present in more than a third of participants are included. The direction and magnitude of the standardized regression coefficient (Std. β) is presented as a colour gradient where blue indicates positive associations and red indicates negative associations. Significance is emphasized with an asterisk: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. All significant results presented are raw *p*-values (not significant with FDR-adjustment).

Free fatty acids associated with cardiometabolic disease risk parameters

The results of the associations between FFAs and CMD risk parameters are presented in **Table S6**. In the fully-adjusted model, 33 FFAs were associated with CMD risk parameters, with the majority (30 FFAs) positively associated with increased CMD risk (raw $p \leq 0.05$). After adjusting for multiple comparisons, associations for 10 FFAs remained significant, which involved primarily long-chain saturated (C16, C17, C18, and C19) and unsaturated FFAs (C17:1 c10, C18:1 c15, C18:2 c9c12, C20:3 c8c11c14, C20:4 c5c8c11c14, and C22:6 c4c7c10c13c16c19) (FDR $p \leq 0.05$) (**Figure 2**). These FFAs were positively associated with plasma total and LDL-C, serum triglycerides, and SCORE; the strongest associations were observed for C18:1 c15 with plasma total (Std. $\beta = 6.3$, $R^2 = 0.3$) and LDL-C (Std. $\beta = 5.6$, $R^2 = 0.2$) (FDR $p \leq 0.05$). Several summed FFAs (total, medium-chain, long-chain, omega-3, omega-6, PUFA, saturated, unsaturated, as well as sum of C18:2 and sum of n-C12, n-C14, and n-C16), were also positively associated with plasma total and LDL-C, or SCORE, albeit with a low magnitude of association (Std. $\beta < 0.1$, FDR $p \leq 0.05$). Sum of C15 and C17, and C15, C17, and C16:1 t9 were also positively associated with total cholesterol (Std. $\beta = 0.8$, FDR $p \leq 0.05$). FFAs negatively associated with CMD risk parameters included C12:1 u with waist circumference, C20, C18.2.t9t12, C18:4 c6c9c12c15, and C18:1 u1 with serum triglycerides, and C18:1 u1 with plasma LDL-C (raw $p \leq 0.05$; non-significant after FDR adjustment) (**Figure S3**).

Figure 3 presents a summary of the individual and summed FFAs that were significantly associated with both dairy intake and CMD risk parameters in the fully-adjuted model. Of these, 5 long-chain unsaturated FFAs (C18.1 t13+c6+c7+u, C18:2 c9t11+u, C20:1 c11, C20:3 c8c11c14, and C20:4 c5c8c11c14), 2 medium-chain saturated FFAs (C15, C15 *iso*), and one medium-chain *trans* FFA (C16:1 t9) were positively associated with at least one variable of dairy intake, as well as plasma total and LDL-C, blood pressure, and SCORE. Interestingly, one long-chain PUFA, C18:2 t9t12, was positively associated with high-fat fermented dairy intake but negatively associated with serum triglyceride levels, while one long-chain saturated FFA, C18:1 u1, was positively associated with cheese intake but negatively associated with plasma LDL-C and serum triglyceride levels.

Associations between dairy intake and cardiometabolic health

The results of the associations between dairy intake and CMD risk factors are presented in **Table S7**. Only a small handful of associations were significant, but these associations attenuated in the final adjusted model (model 3), where only an association between total fermented dairy intake and low-fat fermented dairy intake and SCORE was observed (both: Std. $\beta = 0.2$, $R^2 = 0.2$, raw $p = 0.04$). No associations remained significant after FDR-adjustment.

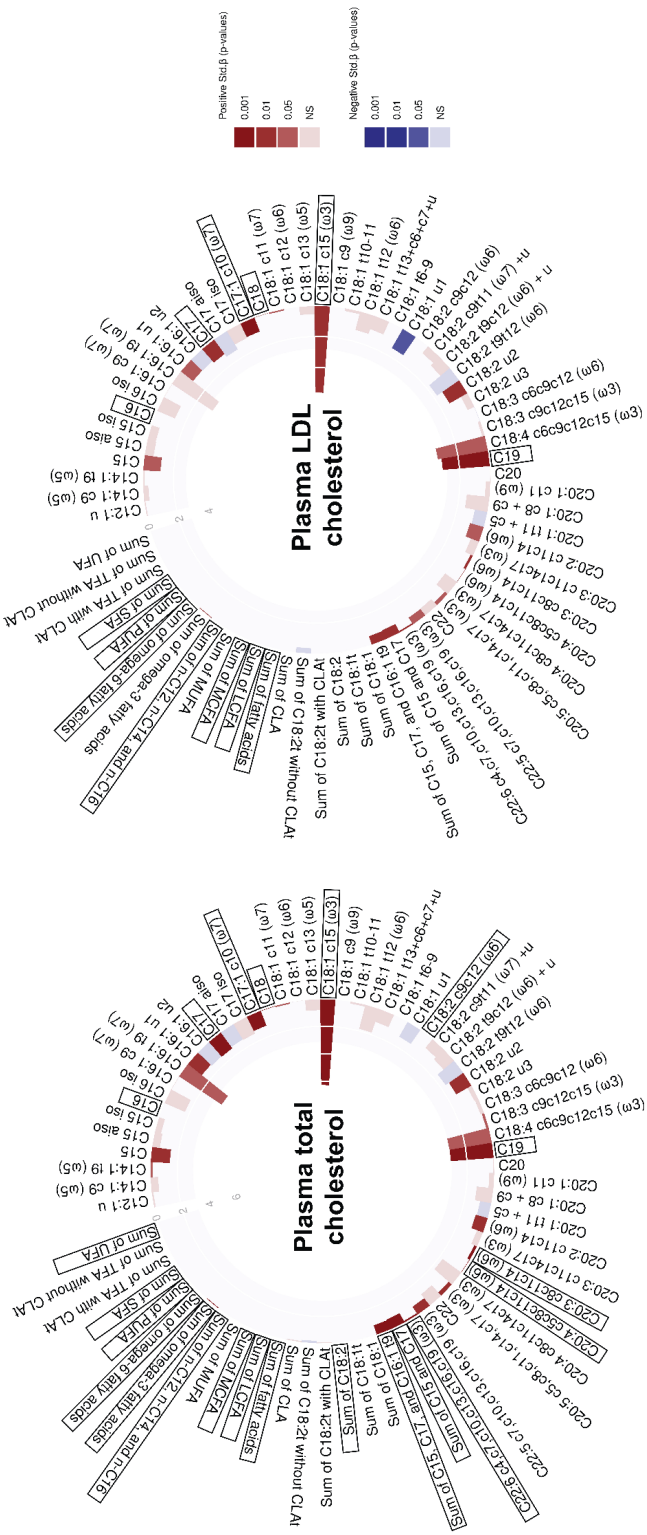

Figure 2. Summary of the associations between FFAs and selected CMD risk factors in the fully-adjusted model (Model 3). Individual and summed FFAs present in more than a third of participants are included. The magnitude of the standardized regression coefficients (Std. β) are indicated in each layer of the circle plot, and the direction and significance of the associations are indicated as a colour gradient. Significant FDR-adjusted associations are boxed in black. LCFA, long-chain fatty acids; LDL, low-density lipoprotein; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids; NS, non-significant; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TG, triglycerides; UFA, unsaturated fatty acids

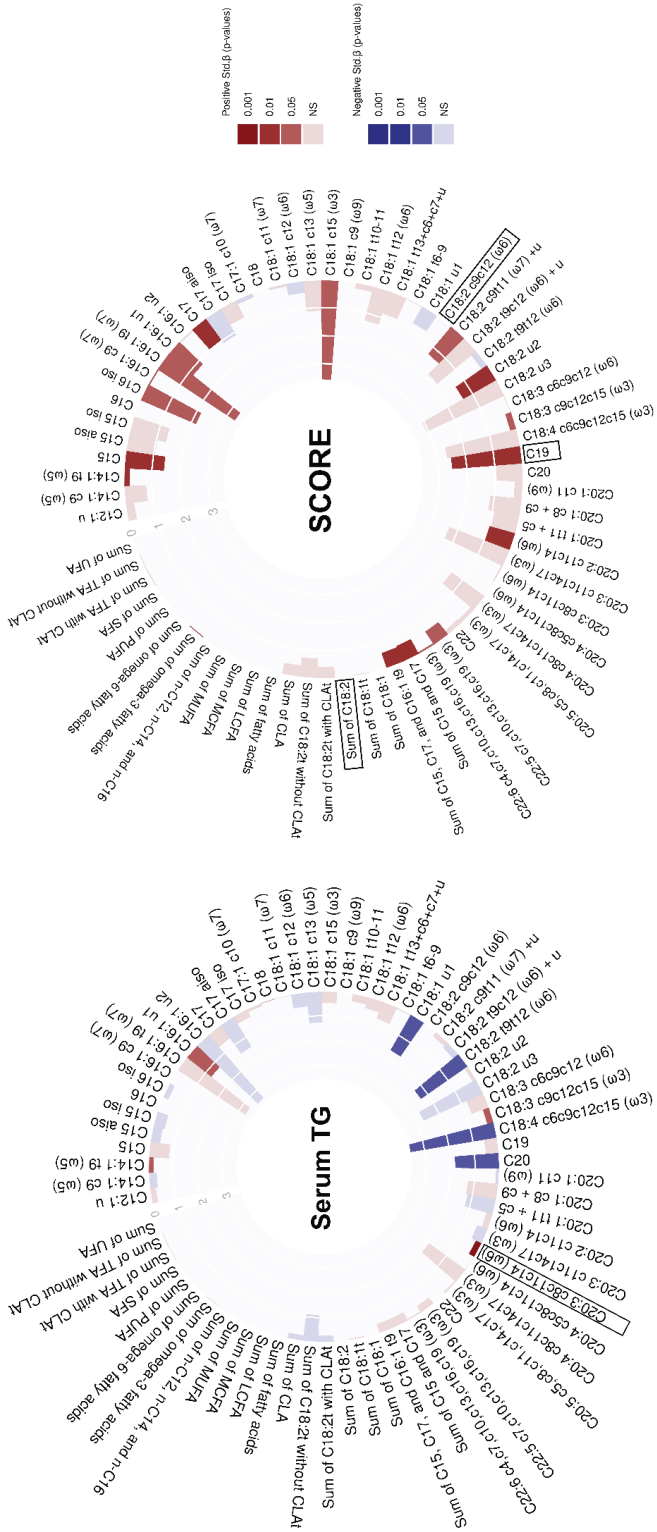

Figure 2 (cont'd).

Figure 3. FFAs significantly associated with both dairy intake and CMD risk parameters in the full-adjusted model (Model 3). Significant positive associations are shown with (●), and significant negative associations are shown with a (●), and significant FDR-adjusted associations are outlined in black ($p \leq 0.05$). BMI, body mass index; DBP, diastolic blood pressure; FD, fermented dairy; HDL, high-density lipoprotein; LC, long-chain; LDL, low-density lipoprotein; MC, medium-chain; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; NFD, non-fermented dairy; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SBP, systolic blood pressure; SCORE, Systematic COronary Risk Evaluation.

Dairy intake groups													Free fatty acids[a]	CMD risk parameters											
Total dairy	Highfat dairy	Lowfat dairy	Dairy fat	Total FD	Highfat FD	Lowfat FD	Total NFD	Highfat NFD	Lowfat NFD	Cheese	Yoghurt	Milk		BMI	Waist circumference	Plasma glucose	Plasma HbA1c	Plasma total cholesterol	Plasma LDL-cholesterol	Plasma HDL-cholesterol	Serum TG	SBP	DBP	Continuous MetS score	SCORE
	●		●										C15 [MC, SFA]					●	●						●
										●			C15 *iso* [MC, SFA]									●	●		
	●		●		●					●			C16:1 t9 (ω7) [MC, MUFA, TFA]					●							●
			●										C16:1 u1 [MC, MUFA]					●	●		●	●	●	●	●
●		●					●		●			●	C17:1 c10 (ω7) [LC, MUFA]					●	●	●					
										●			C18:1 u1 [LC, MUFA]						●		●				
									●				C18:1 c9 (ω9) [LC, MUFA]					●	●			●	●		●
	●							●					C18.1 t13+c6+c7+u [LC, MUFA, TFA]			●									
		●				●							C18:2 t9t12 (ω6) [LC, PUFA, TFA]								●				
			●							●			C18:2 c9t11 (ω7) +u [LC, PUFA]												●
							●	●	●			●	C20:1 c11 (ω9) [LC, MUFA]									●	●		
●		●		●		●			●		●		C20:3 c8c11c14 (ω6) [LC, PUFA]					●	●		●			●	
●		●											C20:4 c5c8c11c14 (ω6) [LC, PUFA]					●	●						
									●				Sum of MUFA					●	●			●	●		●

Discussion

In the current study, we performed a thorough examination of the associations between dairy intake, milk-derived FFAs, and CMD risk parameters. We found that 14 medium- and long-chain FFAs were significantly positively associated with self-reported dairy intake, particularly high-fat dairy intake and dairy fat intake. While some of these FFAs are known to be promising FIBs for dairy fat intake (*e.g.*, C15, C16:1 t9), some of the long-chain unsaturated FFAs found to be associated with dairy intake have not been previously reported in the literature. Concurrently, significant associations were observed between 10 of these FFAs and several CMD risk parameters, particularly plasma total cholesterol and serum triglycerides. The magnitude and robustness of these relationships were maintained and were independent of adjustment for a number of demographic, lifestyle, and dietary factors. These observations suggest that these FFAs could serve a dual role as FIBs of dairy intake and in helping to inform the risk for CMD.

Several fatty acids have been previously proposed as FIBs of dairy intake, including C14, C14:1, C15, C17, C17:1, and C16:1 t9 (54, 55). In particular, the odd-chain fatty acids C15 and C17, which are synthesized by intestinal bacteria in ruminants, have been widely used as indirect measures of dairy fat intake in association studies linking dairy intake to cardiovascular disease, stroke, and type II diabetes (14-17). In our study, we found consistent positive associations between C15 with high-fat dairy and dairy fat intake, and C16:1 t9 with high-fat dairy, dairy fat, high-fat fermented dairy, and cheese intake (no significant associations were observed for C14, C14:1, C17, or C17:1). Significant associations observed for C18:2 c9t11+u (a CLA found in animal fats) with dairy fat and cheese intake confirmed previous associations between this fatty acid with dairy fat intake (56). Conversely, we also observed associations for a number of FFAs that have not previously been considered as FIBs for dairy intake, specifically the branched chain fatty acid C17 *iso* and the *trans* fatty acid C18:1 t13+c6+c7+u for high-fat dairy and dairy fat intakes. A few FFAs were also found to be positively associated with low-fat dairy groups (C22) and specific dairy foods (C15 *iso*, C18:1 u1, C18:2 u3 for cheese, and C20:4 c8c11c14c17 for yoghurt). In addition, although all of the evaluated FFAs have been shown to be present in milk and should theoretically align with (self-reported) dairy intake, we found inverse associations between several FFAs and dairy fat intake, including C16:1 u1, C17:1 c10, C18:3 c6c9c12, and C20:1 c11. This finding illustrates a key conundrum in the identification and validation of FIBs, whereby metabolites found ubiquitously in multiple food sources could challenge their specificity as FIBs for dairy (57). To address this, we have recently shown that a multi-marker comprising multiple metabolites (*i.e.*, a combination of a dairy fatty acid, a non-fatty acid dairy metabolite, and a metabolite reflecting physiological characteristics) may produce a more robust assessment of dairy food intake (30). Nonetheless, identifying which fatty acids are strongly associated with the intake of specific dairy foods is useful for considering their inclusion in multi-marker panels that could help improve the dietary assessment of distinct dairy foods and dairy sub-groups.

An important piece of insight gained in the current analysis is the combined effect of analytical method and classification of 'high-fat' dairy on the strength of the associations observed. Compared to our previous study where we evaluated the robustness of plasma C15 and C17 as FIBs of dairy intake study in a larger subset of the same population (30), stronger correlations were observed for the same evaluations in the current study. The improved correlations may be partly attributed to the quantitative rather than semi-quantitative approach used for the measurement of plasma free fatty acids. In particular, while we previously observed non-significant correlations between C15 with high-fat dairy and high-fat non-fermented dairy groups, these correlations were significant and positive in the current study. Additionally, we observed stronger associations between several FFAs and absolute dairy fat intake, rather than high-fat dairy intake. This could be attributed to the method of classification of dairy foods as 'high-fat': based on what is considered a high fat content for each dairy food (as commonly used for dairy food classification) versus based on the total fat content in the

dairy food. Thus, careful consideration is needed for the classification of 'high-fat' dairy intake in studies where dairy biomarkers are evaluated.

Our study additionally contributes to the current state of the knowledge of using plasma FFAs as reliable biomarkers of habitual dairy fat intake. To date, there have only been a handful of reports investigating FFAs as potential FIBs of dietary fat compared with total fatty acids or fatty acids from other blood fractions, such as cholesteryl esters or serum phospholipids (58, 59). However, these blood fractions best capture short-term dietary fat intake (past several days or hours), as opposed to long-term intake, which is best captured in erythrocyte membrane or adipose tissue (60, 61). These biosamples can be difficult to process and store (*e.g.*, hemolysis of erythrocytes from whole blood) or are invasive (in the case of adipose tissue) (62). As such, they are typically not readily available in biobanks for large population-based studies. In the current report, we demonstrate that plasma FFA can be reliable FIBs of habitual dairy fat intake with associations comparable to studies measuring total FA or FA in various blood fractions.

Aside from their role as FIBs for dairy, milk-derived FFAs could play a dual role in understanding CMD risk. Of all the nutrient groups in dairy, saturated fats have arguably received the most public health attention, due to their adverse effects on circulating blood lipid profile (3, 4). However, saturated fat is not a homogenous nutrient group, but rather consists of diverse individual fatty acids with distinct functional roles. Subgroups of medium-chain, odd-chain, and very long-chain saturated fatty acids found in dairy have been associated with lower risk of type II diabetes and improved overall metabolic health (63). Levels of the odd-chain fatty acid C15 in plasma or serum have been linked with a higher risk of ischemic heart disease (in women) (64), but lower risk of developing type II diabetes, myocardial infarction, cardiovascular disease, and coronary heart disease (17, 65-67). Plasma C16:1 t9 has been associated with higher LDL-C, but also with an improved metabolic profile, lower triglycerides, fasting insulin, blood pressure, and incident type II diabetes (18-20). In our study, we observed the same heterogenous effects between individual FFAs with different indicators of CMD risk. Our analyses revealed positive associations for ~30 FFAs and negative associations for 5 FFAs with different CMD risk parameters. Notably, 8 FFAs that were also significantly associated with dairy intake (including C15, C16:1 t9) were positively associated with plasma total, LDL-C, serum triglycerides, plasma glucose, blood pressure, continuous MetS, and/or SCORE, while two (C18:2 t9t12, C18:1 u1) were negatively associated with plasma LDL-C and serum triglycerides. These exploratory results suggest that distinct milk-derived FFAs could help inform different aspects of CMD risk and progression. However, causal inferences could not be established (based on the cross-sectional nature of the analyses), and thus further verification of these associations is required.

Further, we did not obtain clear associations between self-reported dairy intake and the CMD risk parameters evaluated. All of the associations were neutral and non-significant, aside from a weak positive association between total and low-fat fermented dairy intake and SCORE. The lack of significant associations could reflect the current state of the literature where, collectively, studies examining associations between dairy intake and CMD risk have produced neutral outcomes (68). Here, the use of FIBs for dairy may better relate to the CMD outcomes by accounting for an aspect of individual variability. Evidently, examining the health impacts of a single (dairy) food or food group on CMD also has its limitations, whereas examining their intake in the context of a healthy dietary pattern could offers a stronger explanation of diet-health relations. The lack of associations may also be explained by several study limitations. Our study population was small and relatively healthy, and a larger population with greater variation in CMD risk may have afforded more power to associate the dietary intake of dairy foods to CMD risk parameters. Additionally, based on the data available, we relied on a window of ±14 days between biosample collection and the completion of an FFQ. This assumes that dietary intakes the day prior to biosample collection were comparable to the reported intakes within the reference range of the FFQ, but otherwise, would be a source of measurement error. The use of cross-sectional data also assumes that the current diet reflects past dietary exposures that are responsible for

the current disease risk and furthermore that no major dietary changes have initiated following the appearance of early disease risk markers (e.g., weight gain). For these reasons, CMD causality could not be assessed. Notwithstanding, a strength of the current study is our comprehensive evaluation of dairy intake related biomarkers with multiple individual and composite CMD risk outcomes, which allows us to see how different FFAs associate with and impact these outcomes separately.

In conclusion, our study examining associations between dairy intake, milk-derived FFAs, and CMD risk parameters resulted in the identification of a panel of 10 medium- and long-chain FFAs that were dually associated with both dairy intake and CMD risk. The inclusion of these FFAs in future multi-marker panels could help improve the dietary assessment of different dairy foods. Further exploration in additional, larger prospective cohorts would allow the potential mediating role of these FFAs between dairy intake and CMD to be assessed, and could help confirm their role in CMD risk pathways.

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List of abbreviations

BMI, body mass index; CMD, cardiometabolic disease; FDR, false discovery rate; FFA, free fatty acid; FFQ, food frequency questionnaire; FIB, food intake biomarker; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; NDARD, National Dietary Assessment Reference Database; NQplus, Nutritional Questionnaire plus; PUFA, polyunsaturated fatty acid; SCORE; Systematic COronary Risk Evaluation.

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Supplementary Materials

Table S1. Classification of dairy foods from the NQplus food frequency questionnaire

Dairy Food Item	Fermentation Status	Subgroup	Fat Content[a] (g/100g food)	Fat Classification[b]
Buttermilk	Fermented	Buttermilk	0.2	Skim
Low-fat cheese (20+/30+)	Fermented	Cheese	14.1	Semi-skim
Regular cheese (40+)	Fermented	Cheese	23.9	Semi-skim
Regular cheese (48+)	Fermented	Cheese	30.3	Full fat
Cheese as snack	Fermented	Cheese	28.9	Semi-skim
Cheese with hot meal	Fermented	Cheese	28.9	Semi-skim
Fat luxury cheese	Fermented	Cheese	35.1	Full fat
Less-fat luxury cheese	Fermented	Cheese	22.0	Semi-skim
Unknown cheese	Fermented	Cheese	28.5	Semi-skim
(Fruit) quark with breakfast	Fermented	Quark	2.5	Skim
Full (fruit) yogurt	Fermented	Yogurt	2.8	Full fat
Full yogurt	Fermented	Yogurt	2.8	Full fat
Semi-skim (fruit) yogurt	Fermented	Yogurt	1.5	Semi-skim
Semi-skim yogurt	Fermented	Yogurt	1.8	Semi-skim
Skim (fruit) yogurt	Fermented	Yogurt	0.2	Skim
Skim yogurt	Fermented	Yogurt	0.2	Skim
Unknown yogurt	Fermented	Yogurt	1.8	Semi-skim
Butter	Non-fermented	Butter	81.1	Full fat
Skim Butter	Non-fermented	Butter	37.0	Semi-skim
Coffee cream	Non-fermented	Cream	9.4	Full fat
Cream with hot meal	Non-fermented	Cream	34.2	Full fat
Whipped cream	Non-fermented	Cream	14.8	Full fat
Milk-based ice cream	Non-fermented	Ice cream	12.0	Full fat
Diet coffee milk	Non-fermented	Milk	4.2	Full fat
Full chocolate milk	Non-fermented	Milk	2.8	Full fat
Full milk	Non-fermented	Milk	3.5	Full fat
Full-fat milk with breakfast	Non-fermented	Milk	3.5	Full fat
Regular full milk	Non-fermented	Milk	3.5	Full fat
Regular semi-skim milk	Non-fermented	Milk	1.5	Semi-skim
Semi-skim chocolate milk	Non-fermented	Milk	1.4	Semi-skim
Semi-skim coffee milk	Non-fermented	Milk	4.1	Full fat
Semi-skim milk	Non-fermented	Milk	1.5	Semi-skim
Semi-skim milk with breakfast	Non-fermented	Milk	1.5	Semi-skim
Skim chocolate milk	Non-fermented	Milk	0.5	Skim
Skim milk	Non-fermented	Milk	0.1	Skim
Unknown chocolate milk	Non-fermented	Milk	1.0	Semi-skim
Unknown coffee milk	Non-fermented	Milk	7.3	Full fat
Unknown milk	Non-fermented	Milk	1.4	Semi-skim
Milk powder for coffee	Non-fermented	Milk, powder	32.3	Full fat

[a] The fat content (g/100g) for all dairy products was determined based on the values reported in the Dutch Food Composition Table 2011 (Available from: https://nevo-online.rivm.nl/).

[b] Fat classification was based on the guidelines set by the Dutch Dairy Commodities Act (Overheid.nl. Warenwetbesluit Zuivel), where full-fat dairy included milk and milk products with a fat content >1.80%, cheeses with a fat content ≥50%, and curd cheese/quark and cream cheese with a fat content ≥35%, semi-skim dairy included milk and milk products with a fat content ≥1.50% to ≤1.80%, cheeses with a fat content >10% to <50%, and curd cheese/quark and cream cheese with a fat content ≥10% to ≤34%, and skim dairy included milk and milk products with a fat content ≤0.5%, cheeses with a fat content ≤10%, and curd cheese/quark and cream cheese with a fat content <10%. Additional qualifiers for determining the fat content of Dutch cheeses (based on fat content dry matter) included: full-fat cheese (45+ to 60+), semi-skim cheese (10+ to 40+), and skim cheese (≤10).

Table S2. Summed free fatty acid groups	
Groups	**Individual free fatty acids in group**
Total fatty acids	C4 + C5 + C6 + C7 + C8 + C9 + C10 + C10:1 (ω1) + C12 + C12:1 u + C13 *iso* + C14 + C14 *iso* + C14:1 t9 (ω5) + C14:1 c9 (ω5) + C15 + C15 *iso* + C15 *aiso* + C16 + C16 *iso* + C16:1 t9 (ω7) + C16:1 c9 (ω7) + C16:1 u1 + C16:1 u2 + C17 + C17 *iso* + C17 *aiso* + C17:1 c10 (ω7) + C18 + C18 *iso* + C18:1 u1 + C18:1 t6-9 + C18:1 t10-11 + C18:1 t12 (ω6) + C18:1 t13+c6+c7+u + C18:1 c9 (ω9) + C18:1 c11 (ω7) + C18:1 c12 (ω6) + C18:1 c13 (ω5) + C18:1 c15 (ω3) + C18:2 t10c12 (ω6) + C18:2 t9c12 (ω6)+u + C18:2 t9t12 (ω6) + C18:2 c9c12 (ω6) + C18:2 c9t11 (ω7)+u + C18:2 t9t11 (ω7) + C18:2 c9c11 (ω7)+u + C18:2 u1 + C18:2 u2 + C18:2 u3 + C18:3 c6c9c12 (ω6) + C18:3 c9c12c15 (ω3) + C18:4 c6c9c12c15 (ω3) + C19 + C20 + C20:1 t11 + c5 + C20:1 c8 + c9 + C20:1 c11 (ω9) + C20:2 c11c14 (ω6) + C20:3 c8c11c14 (ω6) + C20:3 c11c14c17 (ω3) + C20:4 c5c8c11c14 (ω6) + C20:4 c8c11c14c17 (ω3) + C20:5 c5,c8,c11,c14,c17 (ω3) + C22 + C22:5 c7,c10,c13,c16,c19 (ω3) + C22:6 c4,c7,c10,c13,c16,c19 (ω3)
SCFA	C4 + C5 + C6 + C7 + C8 + C9 + C10 + C10:1
MCFA	C12 + C12:1 u + C13 *iso* + C14 + C14 *iso* + C14:1 t9 + C14:1 c9 (ω5) + C15 + C15 *iso* + C15 *aiso* + C16 + C16 *iso* + C16:1 t9 + C16:1 c9 + C16:1 u1 + C16:1 u2
LCFA	C17 + C17 *iso* + C17 *aiso* + C17:1 c10 (ω7) + C18 + C18 *iso* + C18:1 u1 + C18:1 t6-9 + C18:1 t10-11 + C18:1 t12 (ω6) + C18:1 t13+c6+c7+u + C18:1 c9 (ω9) + C18:1 c11 (ω7) + C18:1 c12 (ω6) + C18:1 c13 (ω5) + C18:1 c15 (ω3) + C18:2 t10c12 (ω6) + C18:2 t9c12 (ω6)+u + C18:2 t9t12 (ω6) + C18:2 c9c12 (ω6) + C18:2 c9t11 (ω7)+u + C18:2 t9t11 (ω7) + C18:2 c9c11 (ω7)+u + C18:2 u1 + C18:2 u2 + C18:2 u3 + C18:3 c6c9c12 (ω6) + C18:3 c9c12c15 (ω3) + C18:4 c6c9c12c15 (ω3) + C19 + C20 + C20:1 t11 + c5 + C20:1 c8 + c9 + C20:1 c11 (ω9) + C20:2 c11c14 (ω6) + C20:3 c8c11c14 (ω6) + C20:3 c11c14c17 (ω3) + C20:4 c5c8c11c14 (ω6) + C20:4 c8c11c14c17 (ω3) + C20:5 c5,c8,c11,c14,c17 (ω3) + C22 + C22:5 c7,c10,c13,c16,c19 (ω3) + C22:6 c4,c7,c10,c13,c16,c19 (ω3)
Saturated fatty acids	C4 + C5 + C6 + C7 + C8 + C9 + C10 + C12 + C13 *iso* + C14 *iso* + C14 + C15 *iso* + C15 *aiso* + C15 + C16 *iso* + C16 + C17 *iso* + C17 *aiso* + C17 + C18 *iso* + C18 + C19 + C20 + C22
n-C12, n-C14 & n-C16	C12 + C14 + C16
C18:1	C18:1 t6-9 + C18:1 t10-11 + C18:1 t12 + C18:1 t13+c6+c7+u + C18:1 c9 + C18:1 c11 + C18:1 c12 + C18:1 c13 + C18:1u1 + C18:1 c15
C18:2	C18:2 u1 + C18:2 t9t12 + C18:2 u2 + C18:2 u3 + C18:2 t9c12+u + C18:2 c9c12 + C18:2 c9t11+u + C18:2 t10c12 + C18:2 c9c11+u + C18:2 t9t11
Unsaturated fatty acids	C10:1 + C12:1 u1 + C12:1 u2 + C14:1 t9 + C14:1 c9 + C16:1 t9 + C16:1 u1 + C16:1 c9 + C16:1 u2 + C17:1 c10 + C18:1 t6-9 + C18:1 t10-11 + C18:1 t12 + C18:1 t13+c6+c7+u + C18:1 c9 + C18:1 c11 + C18:1 c12 + C18:1 c13 + C18:1 u1 + C18:1 c15 + C18:2 u1 + C18:2 t9t12 + C18:2 u2 + C18:2 u3 + C18:2 t9c12+ u + C18:2 c9c12 + C18:3 c6c9c12 + C20:1 t11+c5 + C20:1 c8+c9 + C20:1 c11 + C18:3 c9c12c15 + C18:2 c9t11+u + C18:2 + C18:2 c9c11+u + C18:2 t9t11 + C18:4 c6c9c12c15 + C20:2 c11c14 + C20:3 c8c11c14 + C20:3 c11c14c17 + C20:4 c5c8c11c14 + C20:4 c8c11c14c17 + C20:5 c5,c8,c11,c14,c17 + C22:5 c7,c10,c13,c16,c19 + C22:6 c4,c7,c10,c13,c16,c19
MUFA	C10:1 + C12:1u1 + C12:1 u2 + C14:1 t9 + C14:1 c9 + C16:1 t9 + C16:1 u1 + C16:1 c9 + C16:1 u2 + C17:1 c10 + C18:1 t6-9 + C18:1 t10-11 + C18:1 t12 + C18:1 t13+c6+c7+u + C18:1 c9 + C18:1 c11 + C18:1 c12 + C18:1 c13 + C18:1 u1 + C18:1 c15 + C20:1 t11+c5 + C20:1 c8+c9 + C20:1 c11
PUFA	C18:2 u1 + C18:2 t9t12 + C18:2 u2 + C18:2 u3 + C18:2 t9c12+u + C18:2 c9c12 + C18:3 c6c9c12 + C18:3 c9c12c15 + C18:2 c9t11+u + C18:2 t10c12 + C18:2 c9c11+u + C18:2 t9t11 + C18:4 c6c9c12c15 + C20:2 c11c14 + C20:3 c8c11c14 + C20:3 c11c14c17 + C20:4 c5c8c11c14 + C20:4 c8c11c14c17 + C20:5 c5,c8,c11,c14,c17 + C22:5 c7,c10,c13,c16,c19 + C22:6 c4,c7,c10,c13,c16,c19
C18:1t	C18:1 t6-9 + C18:1 t10-11 + C18:1 t12 (ω6) + C18:1 t13+c6+c7+u
C18:2t with CLA	C18:2 t9t12 + C18:2 t9c12+u + C18:2 c9t11+u + C18:2 t10c12 + C18:2 t9t11
CLA	C18:2 c9t11+u + C18:2 t9t11
C18:2t without CLA	C18:2 t9t12 + C18:2 t9c12+u
Trans fatty acids without CLA	C14:1 t9 + C16:1 t9 + C18:1 t6-9 + C18:1 t10-11 + C18:1 t12 + C18:1 t13+c6+c7+u + C18:2 t9t12 + C18:2 t9c12+u + C20:1 t11+c5
Trans fatty acids with CLA	C14:1 t9 + C16:1 t9 + C18:1 t6-9 + C18:1 t10-11 + C18:1 t12 + C18:1 t13+c6+c7+u + C18:2 t9t12 + C18:2 t9c12+u + C20:1 t11+c5 + C18:2 c9t11+u + C18:2 t10c12 + C18:2 t9t11
Omega-3 (ω-3) fatty acids	C18:1 c15 + C18:3 c9c12c15 + C18:4 c6c9c12c15 + C20:3 c11c14c17 + C20:4 c8c11c14c17 + C20:5 c5,c8,c11,c14,c17 + C22:5 c7,c10,c13,c16,c19 + C22:6 c4,c7,c10,c13,c16,c19
Omega-6 (ω-6) fatty acids	C18:1 t12 + C18:1 c12 + C18:2 t9t12 + C18:2 t9c12 + C18:2 c9c12 + C18:3 c6c9c12 + C18:2 t10c12 +C20:2 c11c14 + C20:3 c8c11c14 + C20:4 c5c8c11c14
Sum of C15 and C17	C15 + C17
Sum of C15, C17, and C16:1 t9	C15 + C17 + C16:1 t9

c, *cis*; CLA, conjugated linoleic acid; LCFA, long-chain fatty acid; MCFA, medium-chain fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SCFA, short-chain fatty acid; t, *trans*; u, unknown.

Table S3. Mean concentrations of free fatty acids detected in plasma[a]	
Free fatty acids	**Concentration in plasma (mg/mL)[a]**
Individual free fatty acids	
C12:1 u	0.8 ± 0.5
C14:1 c9 (ω5)	0.3 ± 0.2
C14:1 t9 (ω5)	3.5 ± 1.2
C15	0.7 ± 0.2
C15 *aiso*	0.3 ± 0.2
C15 *iso*	0.2 ± 0.1
C16	75.3 ± 17.7
C16 *iso*	0.2 ± 0.1
C16:1 c9 (ω7)	5.8 ± 2.4
C16:1 t9 (ω7)	0.2 ± 0.1
C16:1 u1	0.8 ± 0.2
C16:1 u2	0.1 ± 0.1
C17	1.1 ± 0.3
C17 *aiso*	0.1 ± 0.1
C17 *iso*	0.4 ± 0.1
C17:1 c10 (ω7)	1.3 ± 0.4
C18	30.8 ± 7
C18:1 c11 (ω7)	4.9 ± 1.6
C18:1 c12 (ω6)	0.3 ± 0.2
C18:1 c13 (ω5)	0.2 ± 0.1
C18:1 c15 (ω3)	0.2 ± 0.1
C18:1 c9 (ω9)	64.5 ± 19.9
C18:1 t10-11	1.3 ± 0.4
C18:1 t12 (ω6)	0.4 ± 0.1
C18:1 t13+c6+c7+u	0.6 ± 0.2
C18:1 t6-9	3 ± 1.4
C18:1 u1	0.2 ± 0.2
C18:2 c9c12 (ω6)	46.2 ± 11.5
C18:2 c9t11 (ω7) +u	0.4 ± 0.2
C18:2 t9c12 (ω6) + u	0.2 ± 0.1
C18:2 t9t12 (ω6)	0.2 ± 0.1
C18:2 u2	0.6 ± 0.2
C18:2 u3	0.1 ± 0.1
C18:3 c6c9c12 (ω6)	0.5 ± 0.2
C18:3 c9c12c15 (ω3)	2.2 ± 0.9
C18:4 c6c9c12c15 (ω3)	0.1 ± 0.1
C19	0.3 ± 0.1
C20	0.2 ± 0.1
C20:1 c11 (ω9)	0.7 ± 0.3
C20:1 c8 + c9	0.3 ± 0.1
C20:1 t11 + c5	0.1 ± 0.1
C20:2 c11c14 (ω6)	0.6 ± 0.3
C20:3 c11c14c17 (ω3)	0.1 ± 0.1

C20:3 c8c11c14 (ω6)	3.5 ± 1.3
C20:4 c5c8c11c14 (ω6)	12.6 ± 4.7
C20:4 c8c11c14c17 (ω3)	0.2 ± 0.1
C20:5 c5,c8,c11,c14,c17 (EPA) (ω3)	1.7 ± 1.0
C22	0.3 ± 0.1
C22:5 c7,c10,c13,c16,c19 (DPA) (ω3)	1.2 ± 0.4
C22:6 c4,c7,c10,c13,c16,c19 (DHA) (ω3)	5.6 ± 2.5
Summed free fatty acids	
Sum of fatty acids	296.3 ± 65.2
Sum of MCFA	88.2 ± 21.0
Sum of LCFA	187.6 ± 43.3
Sum of saturated fatty acids	114.3 ± 25.5
Sum of n-C12, n-C14 & n-C16	79.6 ± 18.7
Sum of C18:1	75.5 ± 22.1
Sum of C18:2	47.9 ± 11.7
Sum of unsaturated fatty acids	161.6 ± 39.1
Sum of MUFA	85.1 ± 24.5
Sum of PUFA	76.4 ± 18.6
Sum of C18:1t	5.3 ± 1.8
Sum of C18:2t with CLA	0.8 ± 0.4
Sum of CLA	0.5 ± 0.3
Sum of C18:2t without CLA	0.4 ± 0.2
Sum of *trans* fatty acids without CLA	6.0 ± 1.9
Sum of *trans* fatty acids with CLA	6.4 ± 1.9
Sum of omega-3 fatty acids	11.5 ± 4.2
Sum of omega-6 fatty acids	64.5 ± 15.6
Sum of C15 and C17	1.8 ± 0.5
Sum of C15, C17, and C16:1 t9	2.0 ± 0.5

c, *cis*; CLA, conjugated linoleic acid; LCFA, long-chain fatty acid; MCFA, medium-chain fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; t, *trans*; u, unknown.

[a] Values presented are mean ± standard deviation. Free fatty acids included in the analyses include those detected in at least one third of participants. Seventeen free fatty acids were not detected, and included: C4, C5, C6, C7, C8, C9, C10, C10:1 (ω1), C12, C13 *iso*, C14, C14 *iso*, C18 *iso*, C18:2 c9c11 (ω7) +u, C18:2 t10c12 (ω6), C18:2 t9t11 (ω7), C18:2 u1, sum of short-chain fatty acids.

Table S4. Spearman's correlations between free fatty acids and dairy intakes [a]

Free fatty acid	Spearman's correlation coefficients (r_s) – Mean energy-adjusted intakes (g/day)												
	Total dairy	High-fat dairy	Low-fat dairy	Dairy fat	Total FD	High-fat FD	Low-fat FD	Total NFD	High-fat NFD	Low-fat NFD	Cheese	Yoghurt	Milk
Individual free fatty acids													
C12:1 u	-0.044	0.070	-0.033	-0.062	-0.002	0.010	0.019	-0.061	0.141	-0.106	0.007	0.021	-0.052
C14:1 t9 (ω5)	-0.047	0.064	-0.054	0.047	0.000	0.009	-0.007	-0.021	0.095	-0.070	0.083	0.003	-0.048
C14:1 c9 (ω5)	0.139	0.144	0.087	0.172	0.231**	0.110	0.167	-0.056	0.094	-0.114	0.213*	0.163	-0.093
C15	0.248**	0.202*	0.172	**0.262****	0.271**	0.109	0.205*	0.115	0.178*	0.038	0.190*	0.216*	0.081
C15 *aiso*	0.094	0.110	0.046	0.103	0.145	0.047	0.091	-0.005	0.079	-0.073	0.093	0.154	-0.036
C15 *iso*	0.056	0.216*	-0.006	0.151	0.123	0.141	0.060	-0.011	0.119	-0.095	0.191*	0.144	-0.047
C16	0.070	-0.012	0.063	0.025	0.133	0.006	0.100	-0.044	-0.077	-0.017	0.084	0.121	-0.029
C16 *iso*	0.246**	0.172*	0.177*	**0.303*****	0.264**	0.128	0.199*	0.127	0.113	0.069	0.244**	0.220*	0.092
C16:1 c9 (ω7)	0.000	0.024	-0.014	-0.002	0.125	0.060	0.071	-0.174*	-0.060	-0.175*	0.112	0.069	-0.179*
C16:1 t9 (ω7)	0.206*	0.211*	0.119	**0.324*****	0.200*	0.179*	0.104	0.153	0.123	0.119	0.256**	0.147	0.140
C16:1 u1	-0.013	-0.056	-0.008	-0.148	0.053	-0.095	0.060	-0.097	-0.038	-0.126	-0.021	0.068	-0.099
C16:1 u2	-0.121	-0.041	-0.103	-0.134	-0.030	-0.071	-0.005	-0.134	0.039	-0.168	-0.097	0.031	-0.138
C17	0.220*	0.114	0.160	0.178*	0.229**	0.029	0.184*	0.120	0.128	0.064	0.102	0.233**	0.114
C17 *aiso*	-0.096	0.052	-0.098	-0.030	-0.060	-0.025	-0.071	-0.048	0.081	-0.106	-0.085	-0.021	-0.064
C17 *iso*	0.224*	0.250**	0.151	**0.352*****	0.239**	0.102	0.177*	0.158	0.281**	0.068	0.246**	0.183*	0.122
C17:1 c10 (ω7)	-0.140	-0.040	-0.138	-0.110	-0.068	-0.022	-0.035	-0.155	-0.105	-0.137	-0.054	-0.042	-0.152
C18	0.039	-0.020	0.042	0.008	0.049	-0.012	0.046	0.001	-0.033	0.029	0.015	0.076	0.023
C18:1 c11 (ω7)	-0.002	-0.012	-0.007	-0.104	0.081	-0.020	0.050	-0.113	-0.059	-0.120	-0.012	0.083	-0.095
C18:1 c12 (ω6)	0.126	0.125	0.087	0.146	0.126	0.012	0.126	0.127	0.158	0.066	0.118	0.181*	0.107
C18:1 c13 (ω5)	0.128	0.140	0.079	0.117	0.169	0.084	0.119	0.045	0.057	-0.005	0.165	0.199*	0.042
C18:1 c15 (ω3)	0.158	0.124	0.104	0.182*	0.153	0.093	0.102	0.107	0.013	0.090	0.120	0.142	0.102
C18:1 c9 (ω9)	-0.103	0.032	-0.128	-0.057	-0.010	0.038	-0.050	-0.169	-0.052	-0.193*	0.038	0.007	-0.169
C18:1 t10-11	-0.058	0.058	-0.098	0.096	-0.067	0.038	-0.109	0.016	0.016	-0.020	0.032	-0.084	0.014
C18:1 t12 (ω6)	0.063	0.044	0.056	0.069	0.045	0.069	0.024	0.087	-0.032	0.100	0.030	0.074	0.092
C18:1 t13+c6+c7+u	0.118	0.123	0.067	0.112	0.161	-0.013	0.127	0.045	0.160	-0.020	0.047	0.124	0.041
C18:1 t6-9	0.088	0.105	0.034	0.144	0.052	0.164	-0.017	0.070	-0.003	0.072	0.098	-0.005	0.077
C18:1 u1	0.032	0.199*	-0.019	0.205*	0.057	0.106	0.015	0.048	0.156	0.002	0.220*	0.104	0.016
C18:2 c9c12 (ω6)	-0.054	-0.083	-0.042	-0.044	-0.024	-0.093	-0.011	-0.063	-0.054	-0.048	-0.009	-0.026	-0.051
C18:2 c9t11 (ω7) +u	0.215*	0.133	0.171	**0.291*****	0.257**	0.080	0.206*	0.086	0.107	0.059	**0.295*****	0.183*	0.061
C18:2 t9c12 (ω6) + u	0.071	0.038	0.047	0.065	0.161	0.035	0.114	-0.025	-0.016	-0.057	0.093	0.172	-0.038
C18:2 t9t12 (ω6)	-0.073	0.177*	-0.111	0.088	-0.023	0.171	-0.079	-0.034	0.038	-0.056	0.062	0.076	-0.042
C18:2 u2	0.102	-0.047	0.099	0.006	0.176*	-0.010	0.176*	-0.034	-0.102	-0.018	-0.072	0.127	-0.021
C18:2 u3	0.142	0.228**	0.069	0.179*	0.167	0.178*	0.085	0.088	0.162	0.058	0.154	0.151	0.072
C18:3 c6c9c12 (ω6)	0.147	-0.094	0.178*	0.033	0.162	-0.069	0.176*	0.034	-0.101	0.086	0.119	0.128	0.029
C18:3 c9c12c15 (ω3)	-0.077	-0.033	-0.080	-0.102	-0.045	-0.041	-0.036	-0.065	-0.037	-0.076	-0.004	-0.036	-0.047

C18:4 c6c9c12c15 (ω3)	0.053	0.041	0.036	0.104	0.073	-0.035	0.092	0.056	0.060	0.003	0.096	0.121	0.019
C19	0.017	-0.107	0.021	-0.065	0.047	-0.046	0.045	-0.038	-0.100	-0.027	-0.131	-0.012	-0.017
C20	-0.187*	-0.031	-0.184*	-0.101	-0.097	-0.078	-0.091	-0.164	-0.035	-0.180*	-0.034	-0.065	-0.175*
C20:1 c11 (ω9)	-0.149	0.039	-0.184*	-0.133	0.001	-0.078	-0.011	-0.206*	0.081	**-0.298*****	-0.077	-0.005	-0.220*
C20:1 c8 + c9	0.042	0.037	0.027	-0.018	0.120	-0.020	0.138	-0.025	0.005	-0.044	0.030	0.103	-0.014
C20:1 t11 + c5	0.027	0.011	0.024	-0.019	-0.024	-0.032	0.017	0.088	-0.017	0.038	0.071	-0.011	0.085
C20:2 c11c14 (ω6)	0.145	0.004	0.119	0.059	0.160	-0.057	0.170	0.066	-0.013	0.005	0.065	0.153	0.047
C20:3 c11c14c17 (ω3)	-0.093	-0.052	-0.072	-0.084	-0.115	-0.042	-0.076	0.012	-0.104	0.021	-0.035	0.000	0.018
C20:3 c8c11c14 (ω6)	0.225*	-0.036	0.236**	0.063	0.174*	-0.013	0.175*	0.132	-0.068	0.193*	0.067	0.200*	0.148
C20:4 c5c8c11c14 (ω6)	0.147	-0.049	0.179*	0.014	0.130	-0.098	0.135	0.129	-0.021	0.155	-0.001	0.121	0.145
C20:4 c8c11c14c17 (ω3)	0.115	-0.065	0.122	0.022	0.144	-0.075	0.161	-0.018	-0.072	0.029	0.121	0.160	-0.004
C20:5 c5,c8,c11,c14,c17 (EPA) (ω3)	-0.015	-0.208*	0.044	-0.144	0.033	-0.263**	0.083	-0.087	-0.070	-0.027	-0.050	-0.024	-0.059
C22	0.246**	0.064	0.239**	0.161	0.246**	0.029	0.226**	0.170	0.004	0.147	0.166	0.240**	0.155
C22:5 c7,c10,c13,c16,c19 (DPA) (ω3)	0.134	-0.090	0.166	-0.002	0.114	-0.181*	0.139	0.102	-0.027	0.124	0.039	0.117	0.119
C22:6 c4,c7,c10,c13,c16,c19 (DHA) (ω3)	0.002	-0.163	0.049	-0.138	0.011	-0.231**	0.052	-0.024	-0.041	0.015	-0.111	0.016	-0.006
Summed free fatty acids													
Sum of C15 and C17	0.242**	0.157	0.172*	0.221*	**0.258****	0.060	0.200*	0.119	0.161	0.050	0.136	0.229**	0.097
Sum of C15, C17, and C16:1 t9	0.252**	0.163	0.181*	0.242**	**0.259****	0.074	0.198*	0.136	0.156	0.074	0.155	0.227**	0.117
Sum of C18:1	-0.096	0.041	-0.123	-0.044	-0.011	0.048	-0.054	-0.155	-0.048	-0.178*	0.042	0.005	-0.154
Sum of C18:1t	0.068	0.103	0.012	0.147	0.036	0.113	-0.026	0.083	0.023	0.054	0.068	-0.017	0.086
Sum of C18:2	-0.046	-0.077	-0.037	-0.036	-0.014	-0.092	-0.002	-0.056	-0.046	-0.044	-0.003	-0.014	-0.045
Sum of C18:2t with CLA	0.094	0.133	0.050	0.221*	0.152	0.083	0.084	0.026	0.077	-0.004	0.231**	0.162	0.003
Sum of C18:2t without CLA	-0.031	0.168	-0.076	0.107	0.047	0.156	-0.020	-0.044	0.050	-0.076	0.103	0.137	-0.062
Sum of CLA	0.170	0.147	0.130	**0.293*****	0.221*	0.073	0.164	0.069	0.132	0.029	**0.305*****	0.173*	0.042
Sum of fatty acids	0.002	-0.011	-0.005	-0.013	0.063	-0.018	0.044	-0.072	-0.040	-0.062	0.041	0.072	-0.063
Sum of long-chain fatty acids	-0.019	-0.021	-0.023	-0.030	0.030	-0.036	0.017	-0.065	-0.039	-0.062	0.025	0.043	-0.054
Sum of medium-chain fatty acids	0.067	0.008	0.053	0.038	0.143	0.023	0.103	-0.059	-0.056	-0.041	0.099	0.127	-0.050
Sum of monounsaturated fatty acids	-0.090	0.040	-0.115	-0.044	0.000	0.051	-0.044	-0.160	-0.051	-0.180*	0.046	0.013	-0.159
Sum of n-C12, n-C14, and n-C16	0.071	-0.003	0.058	0.030	0.138	0.015	0.102	-0.048	-0.069	-0.026	0.086	0.131	-0.034
Sum of omega-3 fatty acids	0.006	-0.172*	0.052	-0.119	0.026	-0.234**	0.065	-0.036	-0.066	0.009	-0.047	0.014	-0.013
Sum of omega-6 fatty acids	0.025	-0.066	0.038	-0.011	0.038	-0.083	0.043	0.002	-0.048	0.023	0.017	0.034	0.015
Sum of polyunsaturated fatty acids	0.015	-0.101	0.038	-0.043	0.032	-0.127	0.048	-0.004	-0.056	0.024	-0.007	0.032	0.011
Sum of saturated fatty acids	0.066	0.002	0.056	0.031	0.117	0.009	0.086	-0.029	-0.046	-0.007	0.072	0.118	-0.015
Sum of *trans* fatty acids with CLA	0.067	0.136	0.000	0.199*	0.062	0.144	-0.026	0.064	0.011	0.038	0.139	0.010	0.064
Sum of *trans* fatty acids without CLA	0.069	0.124	0.003	0.162	0.047	0.127	-0.025	0.075	0.024	0.044	0.093	0.003	0.078
Sum of unsaturated fatty acids	-0.042	-0.020	-0.045	-0.046	0.017	-0.031	0.001	-0.091	-0.048	-0.087	0.027	0.027	-0.081

CLA, conjugated linoleic acid; FD, fermented dairy; LCFA, long-chain fatty acid; MUFA, monounsaturated fatty acid; NFD, non-fermented dairy; PUFA, polyunsaturated fatty acid.

[a] All significant results are indicated with an asterisk: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. Significant results after adjustment for multiple comparisons (FDR-adjusted p ≤ 0.05) are boxed in black.

Table S5. Summary of significant associations between free fatty acids and dairy groups

Free fatty acids	Significant in Model 0, 1, 2, and/or 3 [a]												
	Total dairy	High-fat dairy	Low-fat dairy	Dairy fat	Total FD	High-fat FD	Low-fat FD	Total NFD	High-fat NFD	Low-fat NFD	Cheese	Yoghurt	Milk
Individual free fatty acids													
C14:1 c9 (ω5)		0,1		0,1	0	1					0,1		
C14:1 t9 (ω5)		0,1		0,1		1			0,1		0,1		
C15 *iso*											0,1,3		
C15	0	0,1,2,3		0,1,2,3	0	0,1					0,1	0	
C16 *iso*				0,1							0,1		
C16:1 t9 (ω7)	0	0,1,2,3		0,1,2,3		0,1,2,3					0,1,2,3		
C16:1 u1				3									
C17 *iso*	0,1,2,3	0,1,3		0,1,2,3	0,1,2,3		2,3				0,1	0,1,2,3	
C17	0			0								0	
C17:1 c10 (ω7)	1,2,3		1,2,3					0,1,2,3		0,1,2,3			0,1,2,3
C18:1 c15 (ω3)				0									
C18:1 c9 (ω9)			0,1					0		0,1,3			0
C18:1 t13+c6+c7+u		0,1,2,3							0,1,2,3				
C18:1 t6-9				0,1		0,1							
C18:1 u1											0,1,2,3		
C18:2 c9t11 (ω7) +u				0,1,2,3							0,1,2,3		
C18:2 t9t12 (ω6)			1,2,3			2,3							
C18:2 u2					0								
C18:2 u3						2					2,3		
C18:3 c6c9c12 (ω6)		3							3				
C20:1 c11 (ω9)	0		0,1					0,1,2,3	0,1,2,3	0,1,2,3			0,1,2,3
C20:3 c8c11c14 (ω6)	0,1,2,3		0,1,2,3		0,2,3		0,2,3	0,1		0,1,2,3		0,1,2,3	0,1
C20:4 c5c8c11c14 (ω6)	3		2,3										
C20:4 c8c11c14c17 (ω3)												0,2,3	
C20:5 c5,c8,c11,c14,c17 (EPA) (ω3)		0,1		1		0,1							
C22	0,1,2,3		0,1,2,3		0,2,3		0	0,1,2,3		0,1,2,3		0,1,2,3	0,1,2,3
C22:6 c4,c7,c10,c13,c16,c19 (DHA) (ω3)											2		
Summed free fatty acids													
Sum of C18:1			0,1					0		0,1			
Sum of C18:1t		0,1		0,1		0,1							
Sum of C18:2t with CLA											0,2,3		
Sum of C18:2t without CLA			3			2		3		3			3
Sum of CLA				3									
Sum of monounsaturated fatty acids			1					0		0,1,3			0
Sum of *trans* fatty acids with CLA		0,1		0,1		0,1					0,1		
Sum of *trans* fatty acids without CLA		0,1		0,1		0,1							
Sum of C15 and C17	0	0,1		0,1	0							0	
Sum of C15, C17, and C16:1 t9	0	0,1		0,1	0						0	0	

CLA, conjugated linoleic acid; FD, fermented dairy; MUFA, monounsaturated fatty acid; NFD, non-fermented dairy.

[a] Model 0 (unadjusted); Model 1 (age + sex); Model 2 (model 1 + physical activity + education level + smoking); Model 3 (model 2 + alcohol + vegetables + fruits + meat). Free fatty acids presented are significant in at least one model.

Table S6. Summary of significant associations between free fatty acids and CMD risk factors

Free fatty acids	Significant in Model 0, 1, 2, and/or 3[a]											
	BMI	Waist circumference	Plasma glucose	Plasma HbA1c	Plasma total cholesterol	Plasma LDL-cholesterol	Plasma HDL-cholesterol	Serum TG	SBP	DBP	Continuous MetS score	SCORE
Individual free fatty acids												
C12:1 u	1,2	0,1,2,3	0,1,3									
C14:1 c9 (ω5)		1					0					
C14:1 t9 (ω5)			0,2,3		0,1,2,3	0		0,2,3				0,3
C15 *aiso*									0,1,2,3	0,1,2,3		0
C15 *iso*									0,2,3	0,1,2,3		
C15	1	1			0,1,2,3	0,1,2,3						0,3
C16 *iso*					0				0	0		0,3
C16			0,1		0,1,2,3	0,1,2,3		0,1,2,3	0	0,1,2	0,3	0,3
C16:1 c9 (ω7)			0	0			0,1		0	0,1,2,3		0,3
C16:1 t9 (ω7)					0,1,2,3	0,1,2						3
C16:1 u1					0,2,3	2,3		0,1,2,3	0,1,2,3	0,1,2,3	0,3	0,3
C16:1 u2								2		1		
C17 *aiso*								0				
C17 *iso*					0,1,2							
C17					0,1,2,3	0,1,2,3			0			0,3
C17:1 c10 (ω7)					0,1,2,3	0,1,2,3	0,1,2,3					
C18					0,1,2,3	0,1,2,3	0	0,1,2,3	0	0,1		0,3
C18:1 c11 (ω7)					0,2,3	2,3			0,1,2,3	0,1,2,3	3	0
C18:1 c13 (ω5)									0	0,1		0
C18:1 c15 (ω3)			0,1,2,3	0,2,3	0,1,2,3	0,1,2,3	0					0,3
C18:1 c9 (ω9)			0		2,3	3			0,1,2,3	0,1,2,3		0,3
C18:1 t12 (ω6)					0							
C18:1 t13+c6+c7+u			0,1,2,3									
C18:1 u1						2,3		1,2,3				
C18:2 c9c12 (ω6)					0,1,2,3	0,1,2,3			0,2,3	0,2,3		0,3
C18:2 c9t11 (ω7) +u					0		0					0,3
C18:2 t9c12 (ω6) + u									03	0,1		
C18:2 t9t12 (ω6)							0	0,1,2,3				
C18:2 u2			0,1,2,3	0,2,3	0,2,3	0,1,2,3						0,3
C18:2 u3										0,1		
C18:3 c9c12c15 (ω3)			0,1		0,2,3	0,2		0,1,2,3	0,1,2,3	0	0,3	0,3
C18:4 c6c9c12c15 (ω3)					0,2,3	0,3	0,2	1,2,3	0,1,2,3			0
C19					0,1,2,3	0,1,2,3			0,1	0,1		0,3
C20								2,3		0		

C20:1 c11 (ω9)									0,1,2,3	0,1,2,3		
C20:1 c8 + c9						0	0,1	0				0
C20:2 c11c14 (ω6)					0,2,3	0,2,3			0			0,3
C20:3 c11c14c17 (ω3)	0,1									0		0
C20:3 c8c11c14 (ω6)					0,1,2,3	0,1,2,3		0,1,2,3			0,3	
C20:4 c5c8c11c14 (ω6)					0,1,2,3	0,1,2,3	0	0,1,2				0
C20:4 c8c11c14c17 (ω3)	0,1				0,1	0		0,1	0,1	0		0
C20:5 c5,c8,c11,c14,c17 (EPA) (ω3)	0,1	0,1			0,1,2,3	0,1,3	0,1,2					0
C22					0		0,1					
C22:5 c7,c10,c13,c16,c19 (DPA) (ω3)			0,3	0	0,1,2,3	0,1,3	0	0	0	0,1		0,3
C22:6 c4,c7,c10,c13,c16,c19 (DHA) (ω3)					0,1,2,3	0,1,2,3	0	0		2,3		0,3
Summed free fatty acids												
Sum of C18:1			0,1		2,3	2,3			0,1,2,3	0,1,2,3		0,3
Sum of C18:2					0,1,2,3	0,1,2,3			0,2,3	0,2,3		0,3
Sum of C18:2t with CLA							0			0		
Sum of C18:2t without CLA		2										
Sum of CLA							0					
Sum of fatty acids			0		0,1,2,3	0,1,2,3		0,1	0,1,2,3	0,1,2,3		0,3
Sum of long-chain fatty acids			0		0,1,2,3	0,1,2,3		0	0,1,2,3	0,1,2,3		0,3
Sum of medium-chain fatty acids			0,1	0	0,1,2,3	0,1,2,3		0,1,2,3	0	0,1,2	0,3	0,3
Sum of monounsaturated fatty acids			0,1		2,3	2,3			0,1,2,3	0,1,2,3		0,3
Sum of n-C12, n-C14, and n-C16			0,1		0,1,2,3	0,1,2,3		0,1,2,3	0	0,1,2	0,3	0,3
Sum of omega-3 fatty acids					0,1,2,3	0,1,2,3	0	0,1	0	0		0,3
Sum of omega-6 fatty acids					0,1,2,3	0,1,2,3		0,1,3	0,2,3	0,1,2,3		0,3
Sum of polyunsaturated fatty acids					0,1,2,3	0,1,2,3		0,1,3	0,2,3	0,1,2,3		0,3
Sum of saturated fatty acids			0,1		0,1,2,3	0,1,2,3		0,1,2,3	0	0,1,2	0,3	0,3
Sum of unsaturated fatty acids			0		0,1,2,3	0,1,2,3			0,1,2,3	0,1,2,3		0,3
Sum of C15 and C17	1				0,1,2,3	0,1,2,3						0,3
Sum of C15, C17, and C16:1 t9	1				0,1,2,3	0,1,2,3						0,3

BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SBP, systolic blood pressure; SCORE, Systematic COronary Risk Evaluation.

[a] Model 0 (unadjusted); Model 1 (age + sex); Model 2 (model 1 + physical activity + education level + smoking); Model 3 (model 2 + alcohol + vegetables + fruits + meat). For continuous MetS score: Model 0 (unadjusted); Model 3 (smoking + physical activity + education level + alcohol + vegetables + fruits + meat). For SCORE: Model 0 (unadjusted); Model 3 (physical activity + education level + alcohol + vegetables + fruits + meat).

Table S7. Summary of significant associations between dairy groups and CMD risk factors

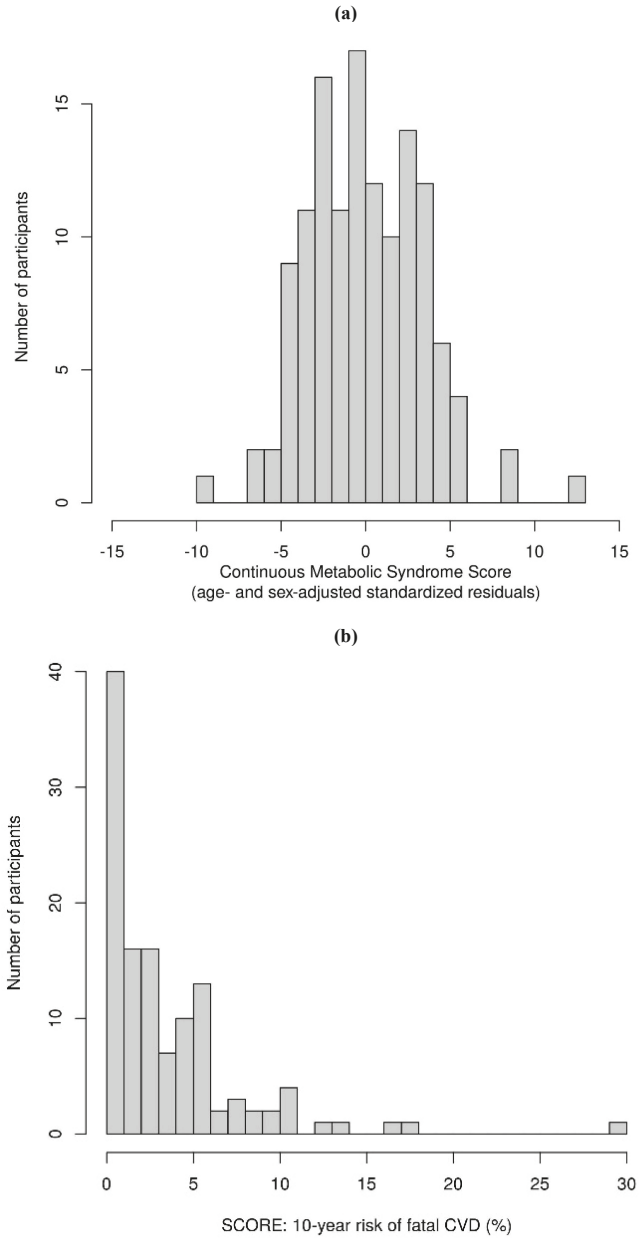
Dairy groups	Significant in Model 0, 1, 2, and/or 3						
	BMI[a]	Waist circumference[a]	Plasma HbA1c[a]	Plasma total cholesterol[a]	Plasma LDL-cholesterol[a]	Serum TG[a]	SCORE[b]
Total FD				0	0		0,3
Low-fat FD			1	0			0,3
Dairy fat	2	2					
Cheese						1	
Yoghurt							

BMI, body mass index; DBP, diastolic blood pressure; FD, fermented dairy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetS, metabolic syndrome; SBP, systolic blood pressure; SCORE, Systematic COronary Risk Evaluation.

[a] Model 0 (unadjusted); Model 1 (age + sex); Model 2 (model 1 + physical activity + education level + smoking); Model 3 (model 2 + alcohol + vegetables + fruits + meat).

[b] Model 0 (unadjusted); Model 3 (physical activity + education level + alcohol + vegetables + fruits + meat).

Figure S1. Distribution of cardiometabolic risk in the population, based on (a) a continuous metabolic syndrome score (age- and sex-adjusted), and (b) 10-year risk of fatal cardiovascular risk based on the European Systematic COronary Risk Evaluation (SCORE) model.



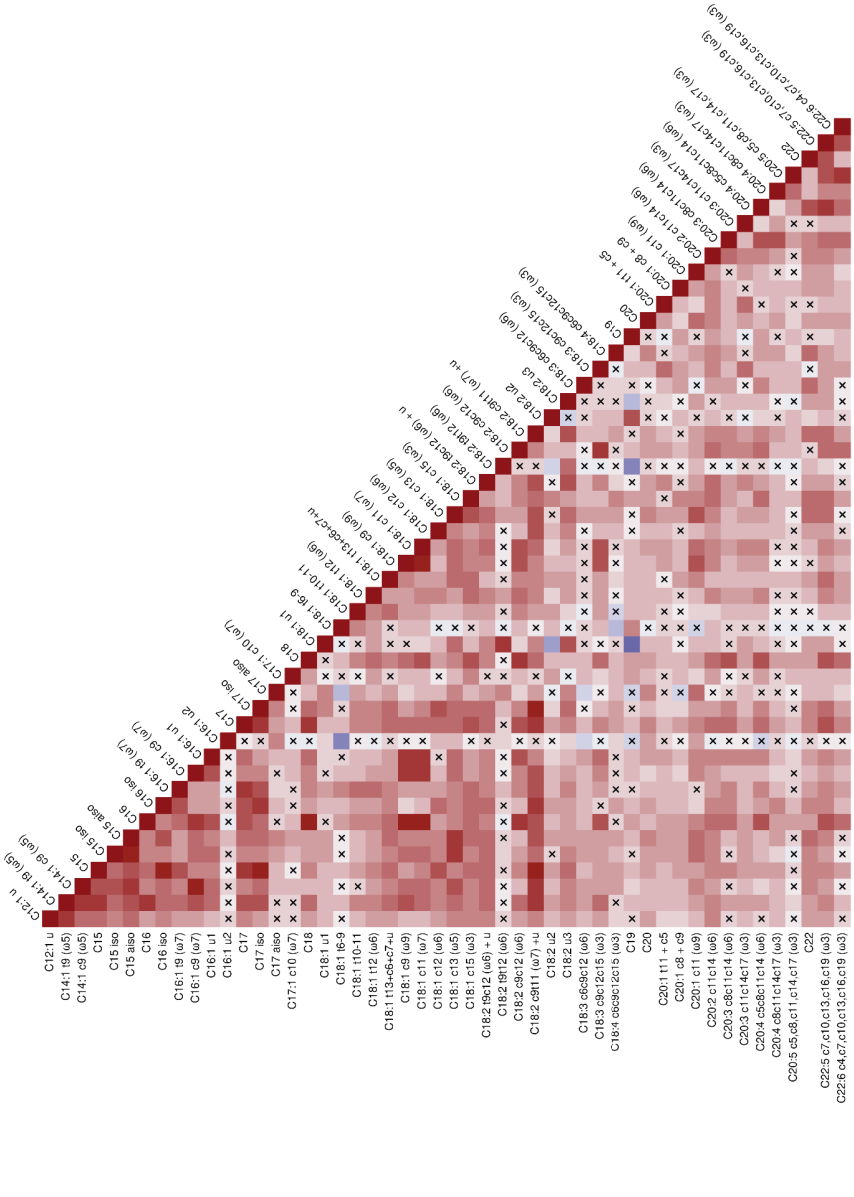


Figure S2. Intercorrelations between free fatty acids. Individual free fatty acids present in more than a third of participants are included. The magnitude of the Spearman's correlation coefficients are represented as a colour gradient, and non-significant correlations (non-FDR adjusted p≥0.05) are indicated with an "x".

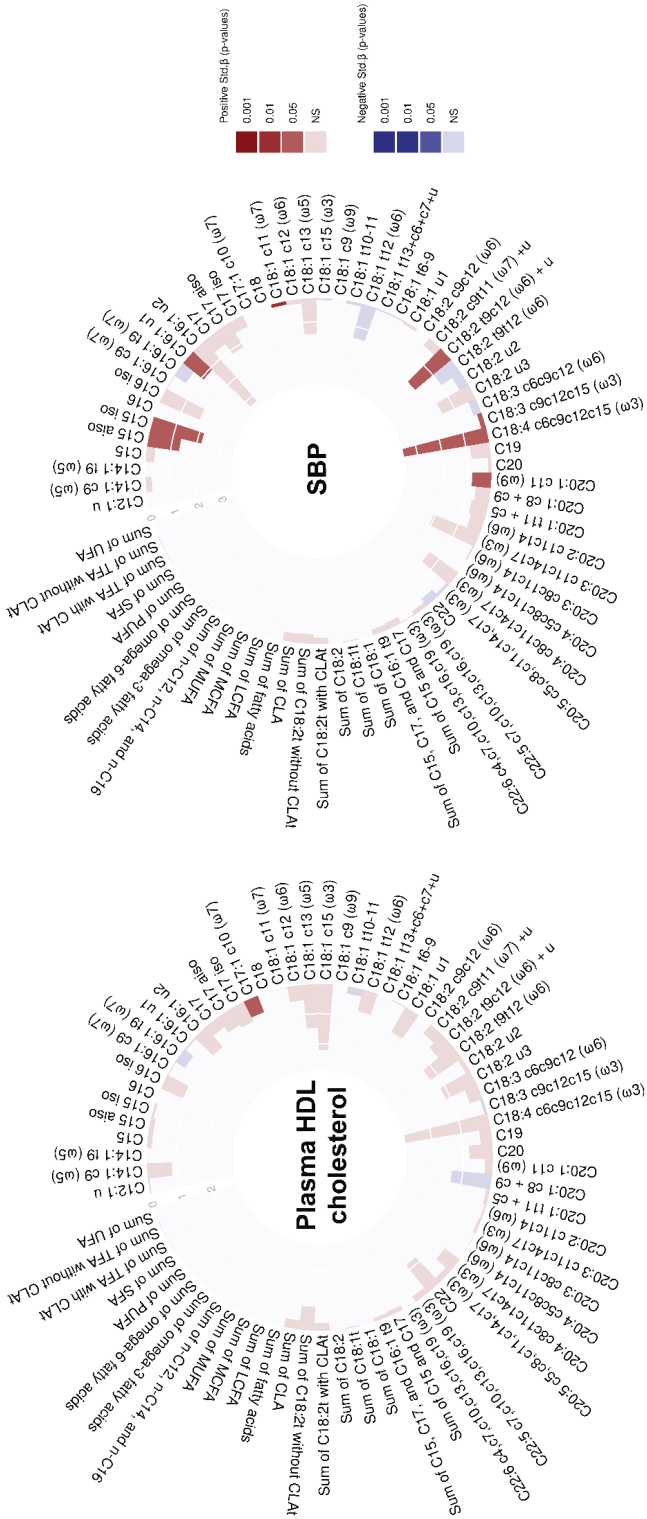


Figure S3. Summary of the associations between free fatty acids and selected CMD risk factors in the fully-adjusted model (Model 3 – adjusted for age, sex, physical activity, education level, smoking, alcohol, vegetables, fruits, meat; for continuous MetS, adjusted for physical activity, education level, smoking, alcohol, vegetables, fruits, meat). Individual and summed free fatty acids present in more than a third of participants are included. The magnitude of the regression coefficients are indicated in each layer of the circle plot, and the direction and significance of the associations are indicated as a colour gradient. All significant results presented are raw *p*-values (not significant with FDR-adjustment). LCFA, long-chain fatty acids; LDL, low-density lipoprotein; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids; NS, non-significant; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TG, triglycerides; UFA, unsaturated fatty acids.

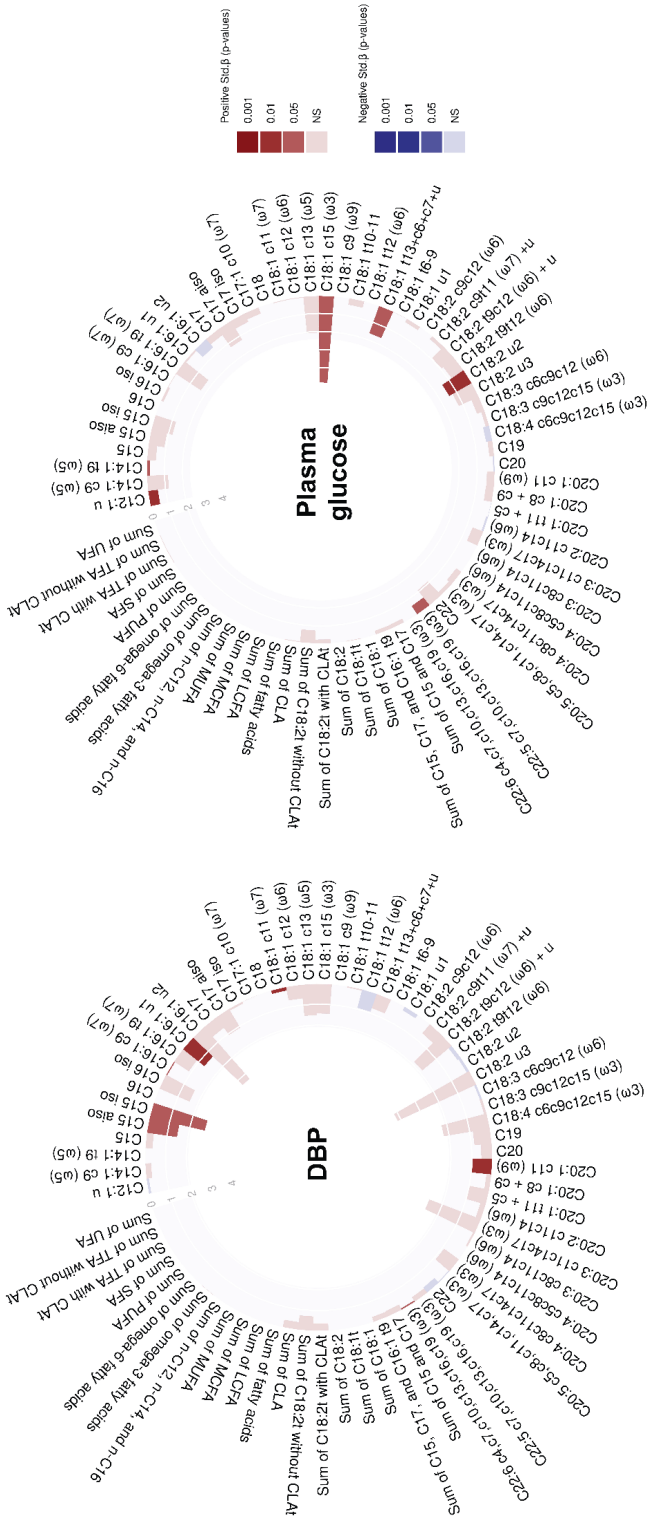


Figure S3 (cont'd).

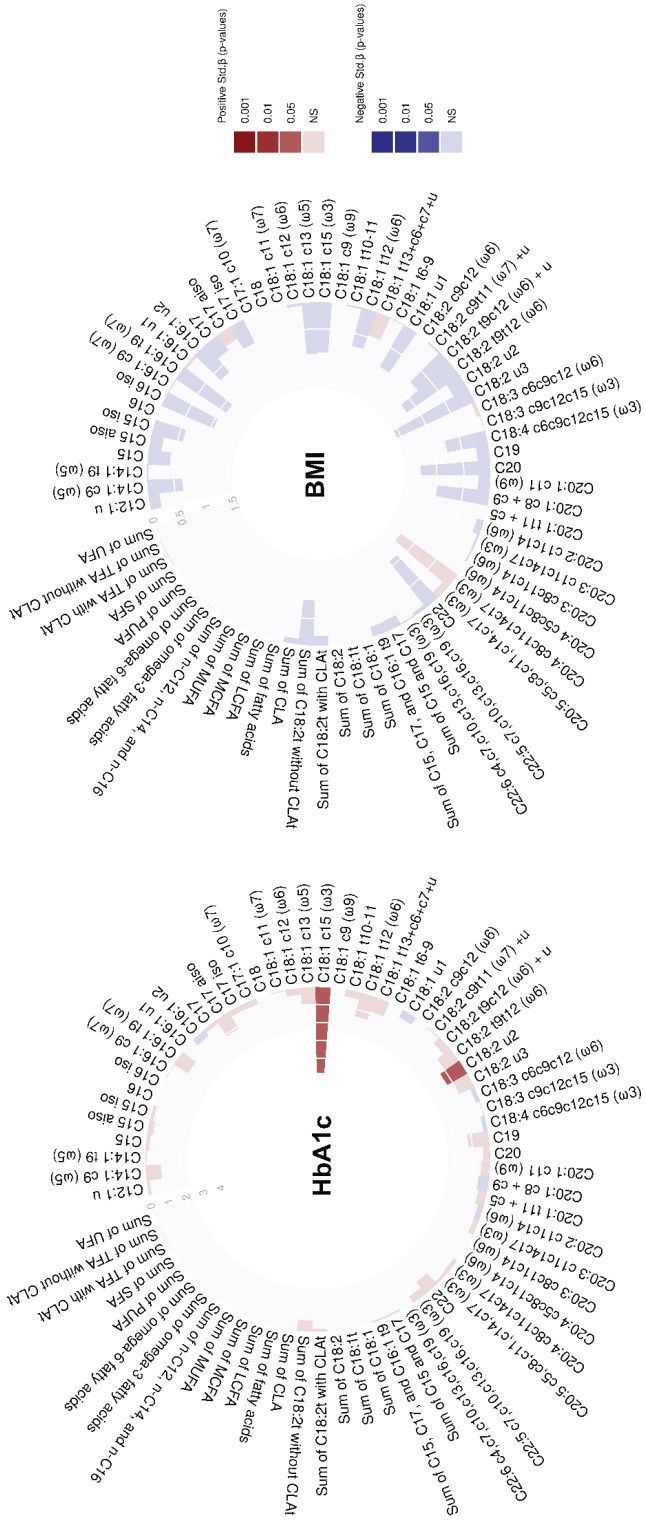


Figure S3 (cont'd).

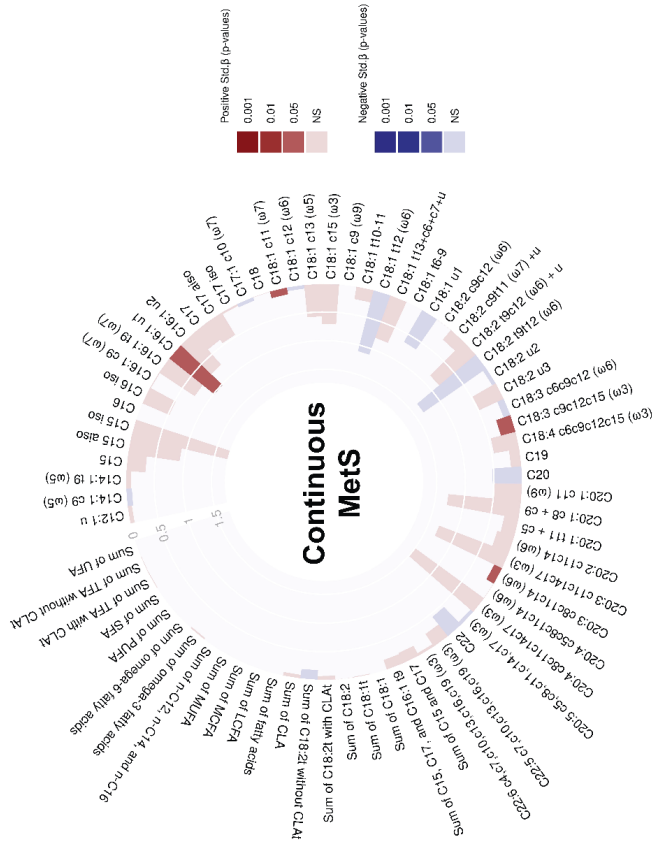


Figure S3 (cont'd).

CHAPTER 6

Identifying plasma and urinary biomarkers of fermented food intake and their associations with cardiometabolic health in a Dutch observational cohort

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In preparation

Abstract

Scope: Food intake biomarkers (FIBs) have the potential to complement self-report tools and improve the accuracy of dietary assessment. Identification of FIBs for fermented foods could help clarify the relationships between the consumption of fermented foods and cardiometabolic health.

Methods and results: We aimed to identify novel FIBs for fermented foods consumed in The Netherlands in 531 free-living participants of the Nutrition Questionnaires plus (NQplus) cohort. Self-reported fermented food intake was obtained from a food frequency questionnaire. The plasma and urine metabolomes of the participants were analyzed using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS). Discriminant metabolites were selected by univariate and multivariate statistical analysis. Further, multivariable adjusted linear regression was used to explore associations between identified metabolites and several cardiometabolic disease risk factors. A total of 36 metabolites were identified in plasma and urine, the majority of which corresponded to the intakes of coffee, wine, and beer (none identified for cocoa, bread, cheese, or yoghurt intake). While some of the identified metabolites appeared to originate from the food raw material (*e.g.*, niacin and trigonelline for coffee, tartaric acid for wine), others overlapped different fermented foods (*e.g.*, 4-hydroxybenzeneacetic acid and ethyl α-D-glucopyranoside for both wine and beer). In addition, several fermentation-dependent metabolites were identified (erythritol and citramalate). Associations between these identified metabolites with cardiometabolic parameters were weak and inconclusive.

Conclusion: The metabolites identified for coffee, beer, and wine intake in this free-living cohort could be considered to be the most robust indicators of habitual fermented food intake. Further evaluation is required in order to confirm their relationships with cardiometabolic disease risk.

Introduction

Accurate dietary assessment is crucial for detecting potential associations between diet and health. To date, many epidemiological studies still predominantly rely on traditional dietary assessment methods, such as food frequency questionnaires (FFQ) and 24-h food recalls. These are self-reported tools that heavily depend on the memory and dedication of the participants (1, 2). As such, they are prone to multiple sources of measurement errors such as underreporting, inaccurate portion size estimation, and imprecision of food composition databases. Such measurement errors can reduce study power and miss detecting potential associations, and may also lead to spurious findings (3, 4). Additionally, to capture the increasing diversity and complexity of modern diets, self-report methods nowadays require extensive food lists, which is burdensome for both participants and researchers. To address these limitations, food intake biomarkers (FIBs) have emerged as an alterative or complementary method of dietary assessment. Since FIBs are detected in biological samples, they are considered to be more objective measures of intake. Depending on their specificity, FIBs can be single compounds or a multi-marker panel consisting of a combination of different compounds (5). Recent advances in nutritional metabolomics has led to the identification of numerous candidate FIBs linked to the ingestion of a food, food group, or a dietary pattern (3, 6). However, FIBs for many foods in the diet have yet to be explored and validated – including fermented foods.

Fermented foods have been consumed since the beginning of human civilization and comprise up to 40% of the human diet (7). They are created through "desired microbial growth and enzymatic conversions of food components" (8). The fermentation process not only improves the shelf-life and organoleptic qualities of a food, it can also impart novel nutritional qualities that could improve human health (9, 10). A number of dietary intervention and epidemiological studies have suggested that consumption of fermented foods positively affect cardiometabolic health, including on weight maintenance, glucose metabolism, and cardiovascular health (9, 11-14), but the evidence is inconclusive. Thus, identification and validation of FIBs for fermented foods could improve the accuracy of dietary assessment, and support further studies in obtaining more conclusive associations between fermented food intake and cardiometabolic health. Additionally, FIBs could also help elucidate the mechanisms of action that underpin the purported health benefits of fermented foods.

We previously conducted a systematic review of FIBs of fermented foods consumed worldwide, and found several candidate FIBs at the food-level, food group-level, and/or fermentation-level for several fermented foods, including wine, beer, bread, cocoa, coffee, post-fermented tea, fermented soy, cheese, and yoghurt (15). The majority of these FIBs were identified in postprandial studies with a small number of participants, and their relevance needs to be explored in free-living populations with complex, uncontrolled diets (16). In the current work, we aimed to identify further FIBs of fermented foods consumed in The Netherlands by analysing the plasma and urine metabolomes of a Dutch adult cohort using liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS). By analysing a larger, free-living population, we expected the FIBs that emerge would be considered to be the most powerful and reliable indicators of habitual fermented food intake. In addition, we examined associations between the identified FIBs and several cardiometabolic risk parameters and composite risk scores.

Experimental Section

Study population

The Nutrition Questionnaires plus (NQplus) study is a prospective cohort study of 2048 Dutch men and women (20 to 70 years) with the aim to gather extensive data on participant demographics, anthropometrics, lifestyle, medical history, and cardiometabolic health outcomes (17, 18). Blood and urine

samples were also collected. Participants were recruited between June 2011 and February 2013. All measurements were performed according to a standardized protocol by trained research personnel. The study was approved by the ethical committee of Wageningen University and Research (protocol number NL34775.081.10) and conducted in agreement with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the start of the study.

Metabolomics analyses were performed on a sub-cohort of NQplus participants (n = 531; n = 485 with plasma samples and n = 492 with urine samples) (herein referred to as the "metabolomics subcohort"). These participants were initially selected based on having a biosample collected within 14 days of completing either a FFQ or a 24-h recall. For the current analysis, the FFQ was preferred over the 24-h recalls, since it reflects more precisely the intake on any given day and is less sensitive to fluctuations in daily intake. Thus, for the selection of the most discriminant metabolites for identification, we focused the analyses on n = 246 unique participants who had a biosample collected within 14 days of completing a FFQ (n = 228 with plasma samples, and n = 216 with urine samples) (herein referred to as the "identification subcohort"). This criterion ensured that biosample collection occurred within the FFQ reference period of one month, which allowed a comparison between the identified FIBs with estimated habitual dietary intake. To explore the stability of the FIBs with increasing time between biosample collection and FFQ completion, additional correlation analyses were conducted among participants with biosample collection within ±30 days (n = 273), ±90 days (n = 354), and ±180 days (n = 501) of completing the FFQ, as well as within all 531 participants in the metabolomics subcohort.

Food frequency questionnaire and levels of fermented food intake

A detailed description of the validated, self-administered, semi-quantitative 216-item FFQ used to assess habitual dietary intake has been reported previously (17, 18). Briefly, participants completed the FFQ online and answered questions relating to frequency by selecting one of ten frequency categories ranging from 'never' to '6–7 days per week' (19). Portion sizes were estimated using commonly used household measures. Total food intake (in g/d) was determined by multiplying consumption frequency by portion size as defined in the Dutch food composition tables (2011) (20). Trained research dieticians conducted several quality checks to ensure the quality of the FFQ assessments. A total of 39 food items were classified as fermented, using criteria described previously (21) (**Table S1**). Most of the fermented foods and food groups in the FFQ have already been judged to have a good agreement with the intakes reported in 24-h recalls (21). Only fermented foods and food groups that achieved 'adequate' to 'good' agreement in the validation study (21) (which is important for determining the reliability of self-reported intakes) and were consumed by at least a third of the population (which is important for the detection of potential FIBs in biosamples and selection of the most relevant FIBs) were included in the current analyses. The fermented foods and food groups included were: fermented beverages (coffee, beer, and wine), fermented cereals/grains (white bread, wholegrain bread), fermented dairy (cheese, yoghurt), and cocoa.

To facilitate the selection of FIBs that reflect the absolute dry weight of the different fermented foods considered within the fermented food groups (beverages, cereals/grains, cocoa-based products, and dairy), we further calculated the g dry matter/day intakes for each fermented food by subtracting the water weight of each food (in g/day) from the total intake (in g/day) (water weight determined from the Dutch food composition tables). Subsequently, energy-adjustment was performed on all individual fermented foods as well as fermented food groups using the commonly used residual method (22). All energy-adjusted fermented food intakes (in g/day and g dry matter/day) were then divided into tertiles representing the low (T1), mid (T2), and high (T3) levels of intake.

Cardiometabolic health parameters

A total of ten cardiometabolic health parameters collected at baseline were included in the current analysis. Details on these cardiometabolic health parameters have been described previously (18). Height was determined using a stadiometer (SECA, Germany, nearest 0.1 cm) and weight was determined using a digital weighing scale (SECA, nearest 0.1 kg). BMI was calculated by dividing weight (in kg) by height (in m^2). Waist circumference was measured twice using a non-flexible measuring tape (SECA 201, nearest 0.5 cm) and averaged. Enzymatic methods (23) were applied to assess fasting plasma glucose, total cholesterol, HDL-cholesterol, and serum triglycerides using Dimension Vista 1500 automated analyser (Siemens, Erlangen, Germany) or Roche Modular P800 chemistry analyser (Roche Diagnostics, Indianapolis, USA). Plasma LDL-cholesterol was calculated with the Friedewald equation (24). Haemoglobin A1c (HbA1c) concentrations in whole blood was determined with HPLC measurement technology using an ADAMS A1c HA-8160 analyser (A. Menarini Diagnostics). Systolic and diastolic blood pressure was measured up to six times using a digital blood pressure monitor (IntelliSense HEM-907, Omron Healthcare, USA); the first measurement was discarded and remaining measurements were averaged. Participants were classified as having hypertension, suboptimal cholesterol, or type II diabetes based on the cut-offs and definitions described in relevant guidelines of the European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) (25-27) and having metabolic syndrome based on the harmonized guidelines of the International Diabetes Federation (IDF) *et al.* (28).

Two composite risk scores were also determined (29). This consisted of a continuous metabolic syndrome (MetS) score determined based on summed age- and sex-adjusted standardized residuals (z-scores) of all individual MetS parameters as risk factors (30-32). Secondly, the 10-year risk of fatal cardiovascular disease was evaluated using the European Systematic COronary Risk Evaluation (SCORE) (low-risk country chart) (33, 34). For the calculation of SCORE, a 'smoker' was defined as current smokers and former smokers who quit >35 years old, and a 'non-smoker' as never smokers and former smokers who quit <35 years old (35).

Covariates

All covariate data relevant to the current work (age, sex, education level, smoking status, physical activity, alcohol consumption, and dietary intake) were collected via questionnaires (18). Participants with no education or primary or lower vocational education as their highest completed education were classified as having a 'low' education level, participants who completed lower secondary or intermediate vocational education were classified as having an 'intermediate' education level, and participants who completed higher secondary education, higher vocational education or university were classified as having a 'high' education level. The definition of a 'smoker' and 'non-smoker' are as outlined above. Information on the participants' usual physical activity over the past four weeks was obtained using the validated Activity Questionnaire for Adults and Adolescents (AQuAA), which provides a the time spent on sedentary, light, moderate and vigorous intensity activities in minutes per week (36). Intake levels of alcohol and different foods were by assessed by a FFQ, as described above. Covariate selection of dietary variables was based on the current scientific literature and statistical testing (described further in the statistical analysis).

LC-MS metabolomics analysis

Ethylenediaminetetraacetic acid (EDTA) plasma and 24-h urine samples collected for NQplus were used for metabolomics analyses. All samples were thawed on ice and kept at 4°C during analysis. Prior to LC-MS analysis, phospholipids were removed from plasma samples to limit ion suppression using the Phree filter (Phenomenex Inc., Torrance, CA). Urine samples were normalized based on the specific gravity as determined by the refractive index (refractometer RE40, Mettler Toledo, Switzerland), as described previously (37, 38).

LC-MS metabolomics analysis was performed using the UltiMate 3000 RS UPLC system (Thermo Fisher Scientific, Waltham, MA) with a Waters Acquity UPLC HSS T3 column (length 150 mm, diameter 2.1 mm, particle size 1.8 µm), coupled with the maXis 4G+ quadrupole time-of-flight mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). A gradient was run from 5% to 95% of mobile phase A within 15 min at 0.4 mL/min. Mobile phase A consisted of Milli-Q water with 0.1% formic acid and mobile phase B consisted of acetonitrile with 0.1% formic acid. The column was heated to 35°C with a post-column cooler set to 25°C. The resulting system pressure was ~600 bar, dependent on the actual composition of the mobile phase at the specific time. The mass spectrometer electrospray interface was operated in positive ion mode and spectra were recorded from 75 to 1500 *m/z*. Collision-induced dissociation was performed using energies from 20 to 70 eV. 5 uL of filtered plasma or normalized urine from each sample were injected once in a randomised sequence. Quality control (QC) pools were prepared from plasma or urine samples by mixing all samples of each sample type at equal volume. QC samples were injected at five sample intervals for signal drift correction. Blanks (consisting of ultrafiltered LC-MS-grade water) were also injected at the beginning and end of each batch for detection of contaminants.

Progenesis QI (v.2.3.6198.24128, NonLinear Dynamics Ltd., Newcastle upon Tyne, United Kingdom) was used for retention time correction, peak-picking, deconvolution, adducts annotation, and normalization (default automatic sensitivity and without minimum peak width). The intensity and the detection limit of the candidate FIBs was also performed by Progenesis QI with the "default" setting. All solvents and reagents for metabolomics analysis were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland).

GC-MS metabolomics analysis

Plasma and urine samples were prepared for GC-MS analysis as described previously for serum (39) and urine (40). Specifically, for each 100 µL plasma sample, 50 µL of an internal standard solution (labelled D-sucrose, 13C12, 98%, Cambridge Isotope Laboratories, Inc., Cambridge, UK, c ≈ 0.16 mg/mL in water) was added, followed by precipitation with 300 µL cold methanol, centrifugation, transfer of supernatant (370 µL), and drying using a vacuum centrifuge. Urine samples were normalized prior to analysis using the refractive index methods described above for the LC-MS analysis. For each 100 µL urine sample, 50 µL of an internal standard solution (labelled D-sucrose) was added and dried using a vacuum centrifuge. The plasma and urine samples further underwent a two-step derivatization (methoximation with O-Methylhydroxylamine hydrochloride followed by silylation with N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA)) and were subjected to analysis by a GC-MS 7890B/MS5977A (Agilent Technologies, Santa Clara, CA, U.S.) with a CombiPAL autosampler (CTC-Analytics AG, Zwingen, Switzerland) and a DB-5 ms fused silica capillary column (60 m, 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies, Basel, Switzerland). The samples were injected using a multimode injector according to the following temperature program: initially 90°C, heating rate 900°C/min until 280°C, held for 5 min and cooled at rate of -30°C/min, and maintained at 250°C. The oven program was as follows: initial temperature 70°C for 2 min, increase up to 160°C at a rate of 5°C/min, increase to 300°C at a rate of 10°C/min, which was held for 36 min, equilibration time 1 min. MS detection mass ranged from 28.5 to 600 Da, MS source temperature was 230°C, and MS Quad temperature was 150°C. Electron ionisation was performed with 70 eV. Each batch was initiated by three injections of QC samples for equilibration and after every 5th plasma sample a fresh QC was injected. At start and end of each batch, a blank sample (milliQ water) was included. QC samples and blank samples underwent the same sample preparation as plasma samples.

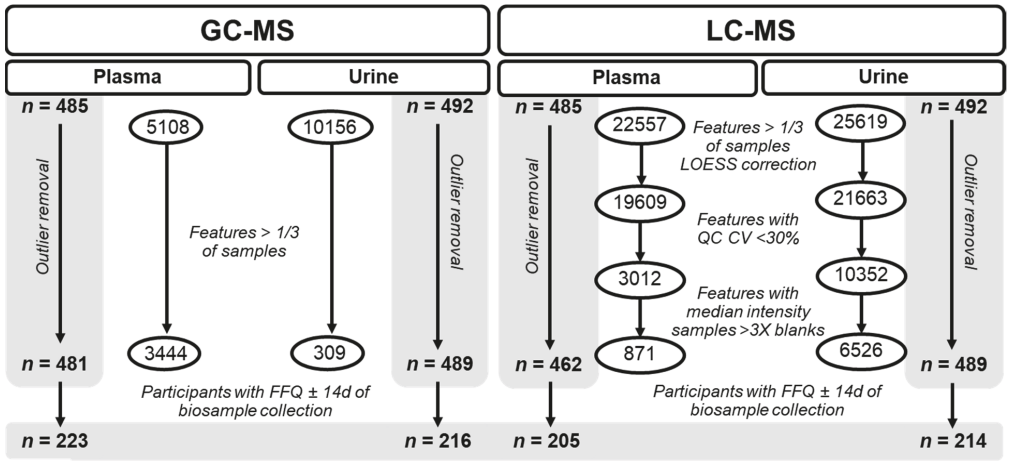
Agilent data files acquired from GC-MS analysis were deconvoluted and converted into CEF files using Agilent MasshunterProfinder (Agilent Technologies, Santa Clara, U.S.). Data files were further processed in Agilent Mass Profiler Professional (Agilent Technologies, Santa Clara, U.S.) to perform alignment and compound identification. In the resulting list containing the deconvoluted features, features with

retention time before 10 min were removed (reagents region). All markers selected based on deconvoluted data were further evaluated using a targeted approach in order to optimize integration. Using RI, quantifier and qualifier ion retrieved from deconvoluted data, the suggested markers were analyzed in MassHunter Quantitative Analysis (Agilent Technologies, Santa Clara, U.S.). The peak integration was checked in each sample individually. Responses from the quantifier ion of marker compounds were normalized with the response of the quantifier ion of internal standard (labelled D-sucrose (ion 220)).

Metabolomics data pre-processing

The dataset was corrected to account for signal drift, and reduced via multiple filtering steps to remove features (metabolites) with poor repeatability and potential contaminants (**Figure 1**). Principal component analyses (PCAs) of the QCs for both LC-MS and GC-MS present the relative stability of the analysis (**Figure S1**). For LC-MS, the QC-based robust locally estimated scatterplot smoothing signal correction method was applied for signal drift correction (41) using R (v.3.6.3) (42). Features resulting from LC-MS analysis were removed if they had poor repeatability (defined as those detected in less than one third of samples), a relative standard deviation > 30 % in the QC samples, and a median in the QC samples that was < 3 times higher than the median calculated for the blanks. For GC-MS, features detected in less than one third of samples were removed (features that had high levels in blanks or originated from the GC column were removed after identification to ensure all features captured during automatic detection are retained and further inspected for relevance). Exploratory analyses were performed and metabolomics sample outliers, defined as observations clearly falling outside Hotelling's T2 tolerance eclipse (95% confidence interval) in the PCA score plot, were identified and excluded (n = 23 LC-MS plasma, n = 3 LC-MS urine, and n = 4 GC-MS plasma, n = 3 GC-MS urine) (**Figure S2**).

Figure 1. GC-MS and LC-MS metabolomics workflow for feature filtration, and outlier removal.



Selection of discriminant metabolites by univariate and multivariate statistics

We performed several complementary univariate and multivariate statistical tests to select and confirm the most consistent signals to proceed with metabolite identification (43). Differences in levels of metabolites by tertiles of intake for fermented foods and food groups (T1, T2, T3) were assessed by a Kruskal-Wallis test followed by a *post-hoc* Conover-Iman pairwise comparison test. An additional step was conducted to select metabolites with higher median levels in higher tertiles compared to lower tertiles (*i.e.*, median of T3 > T1, T3 > T2, and T2 > T1). To determine the strength and direction of the associations between fermented food intakes and metabolites, non-parametric Spearman's rank correlation coefficients (r_s) were calculated; significant correlations with $r_s > 0.20$ were selected for further analysis. For all univariate statistical tests, *p*-values were adjusted for false discovery rate (FDR) using the method of Benjamini and Hochberg (44), and FDR-adjusted $p \leq 0.05$ was set as the significance threshold.

Two multivariate tests were also conducted to further unveil and confirm metabolites that discriminate between tertiles of fermented food intake. Partial least-square discriminant analysis (PLS-DA) was performed to identify metabolites that differentiate the lowest and highest tertiles of intake for each fermented food or food group (SIMCA-P software v.14.0; Umetrics). The dataset was scaled using the unit variance (UV) method. The quality and validity of the models were evaluated by the goodness-of-fit parameter (R2Y > 0.5), the predictive ability parameter (Q2 > 0.2), and permutation tests with 999 random permutations to exclude any random separation of the sample groups (45). Finally, the most discriminant metabolites from these models were selected based on variable importance in projection (VIP) scores (VIP >1 as a cutoff value). Secondly, we used random forests to model these data and further select the most discriminant metabolites between T1, T2, and T3 of fermented food or food group intake, using the randomForest package (46). The dataset was split into training (0.75) and test (0.25) datasets. For tuning the random forest, the number of trees ranged from 500 to 800 and the node sizes range from 1 to 10. The "mtry" parameter was set to *x* (0.01, 0.05, 0.15, 0.25, 0.333, and 0.4), where *x* is the number of features considered for the model. We then implemented a full Cartesian grid-search across the considered tuning parameters to choose the best model using the out-of-bag estimates generated from the random forest model. The results and variable importance from this step were further subjected to permutation testing using the 'altmann' method ($n = 500$) (47) applied in the ranger package (48). For discriminant metabolites selected by multivariate analysis, the Wilcoxon test (for two comparisons) or Kruskal-Wallis test followed by a *post-hoc* Conover-Iman pairwise comparison test (for 3 comparisons) was also conducted (non-FDR adjusted $p \leq 0.05$) as a separate validation test of the metabolites selected from these models.

Given the large number of significant metabolites revealed across complementary univariate and multivariate tests, those significant in at least two of the four statistical tests were prioritized and selected for identification. For urinary metabolites measured by LC-MS, a large number of metabolites remained significant; thus, an additional criterion of Spearman's FDR *p*-value $\leq 1x10^{-10}$ had to be applied to select a number of metabolites that could feasibly be identified. A summary of the significant metabolites across all of the statistical tests (and priortitized for identification) are provided in **Table S2**. Aside from PLS-DA, all analyses were performed in R (v.3.6.3) (42).

Metabolite identification

For LC-MS, the Human Metabolome Database (49), the MassBank of North America (50), and the National Institute of Standards and Technology database (NIST v14), and METLIN (51) were used to screen the identity of metabolites with a 10 ppm mass accuracy threshold. Identity suggestions from databases were then screened based on the chemical and biological relevance of each suggested metabolite identification (as provided on HMDB, and/or through a search of the compound name on PubMed and Google) and confirmed by MS fragmentation data (where available). Pure analytical standards were then purchased for the tentatively

identified and most biologically plausible compounds, and injected at two concentrations in sample QCs and in solvent. For GC-MS, the Golm Metabolome Database (52) and NIST v17 were used to screen the identity of compounds, and an internal database of internal standards was used to confirm the metabolite identification. In the case that stereoisomeric forms of selected discriminating features were identified, the peak with higher response was further evaluated. The list of standards suppliers is provided in **Table S3**. For both LC-MS and GC-MS, the level of identification of each discriminant metabolite is defined according to the Metabolomics Standards Initiative (MSI) recommendations (53), as follows: Level 1, compounds identified by comparison to a pure reference standard based on spectral data (LC: molecular weight with a 10-ppm accuracy threshold, fragmentation pattern when available, isotopic distribution, and retention time with 10% accuracy threshold; GC: based on spectral data and retention indices (RI) with 5% accuracy threshold and 10% for very large peaks); Level 2, based on spectral data but without chemical standards (GC: library match factor >80%); Level 3, putatively characterized compound classes, and; Level 4, unknown compound. Details of the identification features of metabolites analyzed from GC-MS (37 plasma and 75 urinary metabolites) and LC-MS (13 plasma and 89 urinary metabolites) and are presented in **Tables S4** and **S5**, respectively. The metabolites corresponded to the intakes of total fermented beverages (112 metabolites), wine (89 metabolites), coffee (72 metabolites), beer (17 metabolites), white bread (9 metabolites), total fermented cereals/grains (1 metabolite), total fermented dairy (1 metabolite), cheese (1 metabolite), and cocoa (1 metabolite) (none for wholegrain bread or yoghurt).

Associations between identified metabolites and cardiometabolic risk parameters

Participant characteristics are reported as number (percentages), mean (standard deviation) for normally distributed variables, or medians (interquartile range) for skewed variables. Multivariable adjusted linear regression and restricted cubic spline regression were used to evaluate the associations between the identified metabolites and CMD risk factors. The assumption of linear relationships between exposure and outcome variables were tested using likelihood ratio tests of model deviance and Wald tests of spline coefficients. If tests were statistically significant, these associations were visually inspected to confirm the presence of a true non-linear relationship and not due to artificial curves driven by outliers. All tests for non-linearity of the restricted cubic splines revealed that the dose-response association of metabolites and CMD risk parameters could be considered linear. Thus, only linear regressions are presented in the results. CMD risk parameters acting as dependent variables that were not normally distributed were log transformed, which included: BMI, plasma HbA1c, plasma glucose, serum triglycerides, and SCORE. All variables were normalized by z-scores prior to analysis to allow comparability across associations. Analyses were performed unadjusted (Model 0), adjusted for age (years) and sex (male, female) (Model 1) + physical activity (minutes/week), smoking (smoker/non-smoker), and education level (high, intermediate, low) (Model 2) + dietary factors (g/day) (Model 3). For associations with continuous MetS, which already takes into account age and sex, analyses were performed unadjusted (Model 0) and fully-adjusted for smoking, physical activity, education, and dietary factors (Model 3). For associations with SCORE, which already takes into account age, sex, and smoking status, analyses were performed unadjusted (Model 0) and fully-adjusted for physical activity, education, and dietary factors (Model 3). Dietary factors included in the fully-adjusted models included those indicated in the literature to be important for CMD risk in addition to those significantly correlated with the identified metabolites, and included vegetables, fruits, alcohol, meat, and confectionary/desserts. All analyses were performed in R (Version 3.6.3) (54). Visualizations of the associations using circular plots were performed using the ggplot2 R package (55) using a script adapted from Ladroue (56). For all models, the level of significance was set at $p \leq 0.05$. To account for multiple comparisons, FDR-adjusted *p*-values are also presented.

Results

Characteristics of the population

The characteristics of the participants in the metabolomics and identification subcohorts are presented in **Table 1**. The median age of the participants was ~58 years, and the majority were highly educated (>60%) and non-smokers (>69%). No significant differences were observed in background demographics between the two subcohorts. Among the dietary factors, participants in the identification subcohort had significantly higher intakes of total energy, fat, sodium, beer, soft drinks, and egg products compared to participants in the metabolomics subcohort but with a similar interquartile range (significant differences were also observed for tea intake but medians were comparable) ($p \leq 0.05$). Among cardiometabolic parameters, participants in the identification subcohort have a slightly larger waist circumference, higher systolic blood pressure, and lower plasma HDL-cholesterol than participants in the metabolomics subcohort. However, although significant, the differences observed are relatively minor and do not pertain to the broader indicators of health linked to each measure (*e.g.*, BMI, hypertension, suboptimal cholesterol). The distribution of participant risk for continuous MetS and SCORE is presented in **Figure S3**.

Table 1. Characteristics of the study population [a]

Characteristic	Metabolomics subcohort (*n* = 531)	Identification subcohort (*n* = 246)	*p*-value
Demographics			
Age, years	57 (46 - 63)	58 (46 - 65)	0.26
Education, n (%)			0.73
Low	37 (7)	19 (8)	
Intermediate	148 (28)	77 (31)	
High	344 (65)	149 (61)	
Smoking status, n (%)			0.20
Smoker	118 (26)	70 (31)	
Non-smoker	343 (74)	159 (69)	
Physical activity, min/week	2136 ± 1093	2043 ± 1046	0.37
Supplement use, n (%)	0.8 ± 1.2	0.7 ± 1.2	0.58
Dietary factors			
Total energy intake, kcal/day	2128 ± 499	2220 ± 530	**0.02***
Macronutrients			
Fat, g/day (En%)	84 ± 25 (36%)	90 ± 27 (36%)	**0.01***
Carbohydrates, g/day (En%)	230 ± 60 (43%)	237 ± 63 (43%)	0.17
Protein, g/day (En%)	77 ± 18 (14%)	80 ± 18 (14%)	0.06
Fibre, g/day	25 ± 7	25 ± 7	0.80
Sodium, mg/day	2261 ± 653	2375 ± 711	**0.03***
Fermented foods and groups			
Total fermented beverages, g/day	592 (324 - 799)	629 (406 - 865)	0.26
Coffee, g/day	406 (174 - 638)	406 (196 - 638)	0.48
Wine, g/day	25 (4 - 87)	20 (0 - 80)	0.31
Beer, g/day	9 (0 - 79)	20 (0 - 118)	**0.04***
Total fermented cereals/grains, g/day	130 (88 - 166)	133 (88 - 170)	0.41
Wholegrain bread, g/day	80 (47 - 112)	77 (41 - 114)	0.51
White bread, g/day	2 (0 - 8)	2 (0 - 10)	0.24
Cocoa, g/day	4 (1 - 8)	4 (1 - 8)	0.80
Total fermented dairy, g/day	152 (76 - 245)	151 (69 - 240)	0.79
Cheese, g/day	25 (13 - 42)	28 (14 - 46)	0.24
Yoghurt, g/day	89 (29 - 139)	82 (21 - 139)	0.30
Other foods and groups			
Tea, g/day	174 (67 - 406)	174 (67 - 406)	**0.04***
Alcoholic drinks, g/day	81 (18 - 207)	108 (19 - 245)	0.26
Soft drinks, g/day	5 (0 - 42)	13 (0 - 54)	**0.04***
Fruits, g/day	217 (86 - 238)	166 (81 - 233)	0.10
Vegetables, g/day	150 (97 - 204)	140 (94 - 196)	0.11
Potatoes, g/day	67 (37 - 87)	67 (37 - 87)	0.29
Legumes, g/day	38 (19 - 79)	38 (22 - 79)	0.89
Meat products, g/day	72 (46 - 98)	79 (54 - 105)	0.053

Eggs and egg products, g/day	9 (7 - 18)	14 (7 - 18)	**0.03***
Fish, g/day	11 (6 - 16)	11 (6 - 16)	0.72
Nuts and seeds, g/day	13 (6 - 25)	13 (6 - 26)	0.71
Sauces, spreads and cooking fats, g/day	41 (28 - 54)	42 (30 - 57)	0.21
Salty and processed snack foods, g/day	35 (16 - 59)	37 (20 - 64)	0.16
Sugary confectionary and desserts, g/day	70 (47 - 104)	78 (50 - 113)	0.09
Cardiometabolic factors			
BMI, kg/m^2	25.1 (22.9 – 27.2)	25.5 (23.2 – 28.0)	0.12
BMI category, n (%)			0.61
Underweight (<18.5 kg/m^2)	4 (1)	2 (1)	
Normal weight (18.5-24.9 kg/m^2)	249 (7)	103 (42)	
Overweight or obese (≥25-29.9 kg/m^2)	278 (52)	141 (57)	
Waist circumference, cm	91 ± 12	93 ± 12	**0.04***
Diastolic blood pressure, mm Hg	73.7 ± 10.4	74.5 ± 10.8	0.38
Systolic blood pressure, mm Hg	125.5 ± 16.0	128.7 ± 16.6	**0.01***
Hypertension, n (%)			0.42
Hypertension[b]	109 (20.6)	62 (25.2)	
Normal or optimal	421 (79.4)	184 (74.8)	
Hypertension treatment, n (%)			0.95
Being treated with medication and/or diet	69 (13.0)	36 (14.6)	
Not being treated	462 (87.0)	210 (85.4)	
Plasma total cholesterol, mmol/L	5.4 ± 1.0	5.3 ± 1.0	0.15
Plasma LDL-cholesterol, mmol/L	3.3 ± 0.9	3.2 ± 0.9	0.63
Plasma HDL-cholesterol, mmol/L	1.6 ± 0.4	1.5 ± 0.4	**0.01***
Serum triglycerides, mmol/L	1.0 (0.7 – 1.4)	1.0 (0.7 – 1.4)	0.74
Suboptimal cholesterol, n (%)	398 (75.0)	182 (74.0)	0.84
High cholesterol treatment, n (%)			0.76
Being treated with medication and/or diet	56 (10.5)	23 (9.3)	
Not being treated	475 (89.5)	223 (90.7)	
HbA1c, mmol/mol	35.5 (34.0 – 38.0)	35.8 (34.0 – 38.0)	0.97
Fasting glucose, mmol/L	5.4 (5.1 – 5.8)	5.3 (5.0 – 5.8)	0.11
Diabetes, n (%)	13 (2.4)	6 (2.4)	0.98
Diabetes treatment, n (%)			0.77
Being treated with medication and/or diet	15 (2.8)	5 (2.0)	
Not being treated	516 (97.2)	241 (98.0)	
Metabolic syndrome, n (%)	67 (12.6)	33 (13.4)	0.85
SCORE, n (%)			0.25
≥15 %	6 (1.3)	6 (2.6)	
10-14 %	17 (3.7)	14 (6.2)	
5-9 %	73 (16.0)	42 (18.5)	
1-4%	211 (46.2)	98 (43.2)	
<1 %	150 (32.8)	67 (29.5)	

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SCORE, Systematic COronary Risk Evaluation; SD, standard deviation.

[a] Values are presented as mean ± SD, unless otherwise specified. Missing values for the metabolomics subcohort: education (n = 2), smoking (n = 70), physical activity (n = 296), LDL-cholesterol (n = 4), HDL-cholesterol (n = 4), Hb1Ac (n = 5), glucose (n = 4), SCORE (n = 74). Missing values for the identification subcohort: education (n = 1), smoking (n = 17), physical activity (n = 41), LDL-cholesterol (n = 3), HDL-cholesterol (n = 3), Hb1Ac (n = 4), glucose (n = 3), SCORE (n = 19). Differences between the metabolomics and identification subcohorts were assessed using the t-test (for normally-distributed continuous variables), Wilcoxon test (for skewed continuous variables), or chi-squared test (for categorical variables).

[b] Inclusive of Grade 1 hypertension, Grade 2 hypertension, and isolated systolic hypertension.

Intake levels of different fermented foods

The levels of intake of fermented foods in the identification subcohort (mean, tertiles) are presented in **Table 2**. For each fermented food and food group, the levels of intake are presented in both absolute g/day and g dry matter/day. As a consequence of differences in water content, individual fermented foods and fermented food groups with the highest levels of intake differed between the two intake representations. Out of the fermented food groups evaluated, the highest intake on a g/day basis was total fermented beverages followed by total fermented dairy, while the highest mean intake of foods on a g dry/matter per day was total fermented cereals/grains. Out of individual fermented foods, coffee had the highest intakes among all other fermented foods on a g/day basis (466 g/day), but lowest intakes on a g dry matter/day basis (similar trends were observed for wine and beer). Conversely, intakes of cocoa remained the same regardless of g/day or g dry matter/day (similar trends were observed for white and wholegrain bread, and cheese).

Table 2 Tertiles of fermented food intake in the identification subcohort (*n* = 246)

	Energy-Adjusted Intakes (g/day)				Energy-Adjusted Intakes (g dry matter/day)			
Food Group	**Mean ± SD**	**T1 (*n* = 82)**	**T2 (*n* = 82)**	**T3 (*n* = 82)**	**Mean ± SD**	**T1 (*n* = 82)**	**T2 (*n* = 82)**	**T3 (*n* = 82)**
Total FB	638 ± 398	264 (124 , 378)	615 (551, 679)	978 (857 , 1205)	22 ± 20	6 (3, 8)	17 (14, 21)	37 (30, 51)
Coffee	466 ± 297	142 (63, 238)	453 (418 , 510)	691 (640 , 903)	5 ± 3	1 (1, 2)	5 (4, 5)	7 (6, 9)
Beer	112 ± 202	-14 (-32, 11)	48 (37, 71)	208 (136, 374)	9 ± 16	-1 (-3, 1)	4 (3, 6)	17 (11, 30)
Wine	61 ± 89	3 (-7, 7)	25 (16, 38)	130 (92, 187)	8 ± 12	0 (-1, 1)	4 (2, 5)	17 (12, 27)
Total FCG	134 ± 60	79 (61, 94)	132 (115 , 144)	182 (168, 219)	85 ± 37	52 (38, 61)	85 (74, 91)	116 (105, 137)
White bread	8 ± 13	-1 (-2, 1)	4 (3, 5)	17 (10, 30)	5 ± 8	0 (-1, 1)	3 (2, 3)	11 (7, 19)
Wholegrain bread	82 ± 56	29 (12, 42)	77 (68, 88)	131 (114 , 149)	52 ± 35	20 (8, 27)	49 (43, 55)	83 (72, 93)
Cocoa	6 ± 9	1 (0, 1)	3 (3, 4)	10 (8, 17)	6 ± 9	1 (0, 1)	3 (2, 4)	10 (8, 17)
Total FD	170 ± 121	55 (33, 69)	142 (126, 173)	286 (238, 342)	35 ± 19	16 (11, 21)	32 (28, 38)	54 (47, 63)
Cheese	34 ± 26	12 (7, 17)	27 (23, 32)	56 (47, 73)	19 ± 15	7 (4, 10)	16 (13, 18)	31 (27, 42)
Yoghurt	89 ± 79	7 (0, 21)	82 (59, 96)	139 (139, 193)	11 ± 10	1 (0, 3)	11 (8, 13)	20 (17, 24)

FB, fermented beverages; FCG, fermented cereals and grains; FD, fermented dairy. Values are reported as median (IQR), unless otherwise specified.

Biomarkers identified for fermented food intake

A total of 12 plasma metabolites and 26 urinary metabolites were identified. An overview of the candidate FIBs identified for various fermented foods and food groups, along with their platforms and biosamples of detection, are presented in **Table 3** (plasma) and **Table 4** (urine). The majority of the identified metabolites corresponded to the intakes of total fermented beverages (7 plasma, 19 urine), which encompasses coffee (3 plasma, 9 urine), wine (3 plasma, 9 urine), and beer (1 plasma, 6 urine). One urinary metabolite identified was discriminant for the intakes of total fermented cereals/grains, and one plasma metabolite for white bread. However, metabolites discrminant for the intakes of wholegrain bread, cocoa, total fermented dairy, cheese, and yoghurt could not be identified.

A closer examination revealed that several of the identified metabolites (plasma dodecanoic acid, urinary D-psicose, glycine, D-gluconate, m-cresol, D-fucitol, and 2-keto-l-gluconic acid) were negatively associated with the fermented foods and food groups indicated (based on Spearman's correlations, **Table S6**), but contributed to the discrimination of the intake of these fermented foods based on statistical significance in other tests (*e.g.*, PLS-DA, Random Forest). Thus, these metabolites may not be suitable for reflecting fermented food intake and thus not further discussed as FIBs. However, they may still be important biomarkers in revealing the metabolic effects of consuming these fermented foods.

Several of the identified FIBs overlapped across several fermented foods. Specifically, urinary 2,3-dihydroxybutanoic acid, ethyl α-D-glucopyranoside, and 4-hydroxybenzeacetic acid were discriminant for the intake of total fermented beverages, wine, and beer. Several other urinary metabolites also appeared to overlap between two fermented food groups, including 2,3-dihydroxypropyl phosphoric acid and D-lactose (total fermented beverages and beer), catechol, furoylglycine, niacin, and 3-deoxy-D-ribo-hexonic acid gamma-lactone (total fermented beverages and coffee), as well as erythritol, tartaric acid, and arabinofuranose for total fermented beverages and wine. However, in each case, the significance in the total fermented beverages group could be driven by the significance of the individual beverages belonging to this fermented food group. Similar overlaps were observed in plasma for L-cysteine (total fermented beverages and beer), xylitol (total fermented beverages and wine), and quinate (total fermented beverages and coffee). One metabolite identified for wine intake (erythritol) was identified in both plasma and urine.

Table 3. Overview of identified plasma metabolites discriminant for the intake of various fermented foods

				Fermented food or food group										
Identification	**HMDB ID**	**Platform**	**ID level**	**Total FB**	**Coffee**	**Wine**	**Beer**	**Total FCG**	**WG bread**	**WT bread**	**Cocoa**	**Total FD**	**Cheese**	**Yoghurt**
Erythritol	HMDB0002994	GC-MS	1			●								
2-Hydroxybutyric acid	HMDB0000008	GC-MS	1	●										
L-Cysteine	HMDB0000574	GC-MS	1	●			●							
Dodecanoic acid[a]	HMDB0000638	GC-MS	1							●				
Xylitol	HMDB0242149	GC-MS	1	●		●								
trans-Aconitic acid	HMDB0000958	GC-MS	1	●										
Quinate	HMDB0003072	GC-MS	1	●	●									
L-Phenylalanine	HMDB0000159	GC-MS	1	●										
Isoleucine	HMDB0000172	GC-MS	1	●										
Glutamic acid	HMDB0000148	LC-MS	1[b]		●									
Trigonelline	HMDB0000875	LC-MS	1[b]		●									
Hydroxy(*iso*)butyric acid	HMDB0000023	LC-MS	2			●								

FB, fermented beverages; FCG, fermented cereals and grains; FD, fermented dairy; GC-MS, gas chromatography mass spectrometry; ID, identification; LC-MS, liquid chromatography mass spectrometry; WG, wholegrain; WT, white.

[a] Based on the Spearman's correlations this metabolite is negatively associated with the fermented foods and food groups indicated, but discriminant based on statistical significance in other tests (*e.g.*, PLS-DA, Random Forest).

[b] Matched on retention time and mass; information on fragmentation not available.

Table 4. Overview of identified urine metabolites discriminant for the intake of various fermented foods

				Fermented food or food group										
Identification	**HMDB ID**	**Platform**	**ID level**	**Total FB**	**Coffee**	**Wine**	**Beer**	**Total FCG**	**WG bread**	**WT bread**	**Cocoa**	**Total FD**	**Cheese**	**Yoghurt**
D-Psicose[a]	HMDB0250793	GC-MS	1	●	●		●							
Glycine[a]	HMDB0000123	GC-MS	1	●										
D-Gluconate[a]	HMDB0000625	GC-MS	1			●								
Guaiacol	HMDB0001398	GC-MS	1	●										
3-deoxy-D-ribo-hexonic acid gamma-lactone	Not available	GC-MS	2	●	●									
2,3-Dihydroxypropyl phosphoric acid	Not available	GC-MS	2	●			●							
Glucuronic acid	HMDB0000127	GC-MS	2			●								
m-Cresol[a]	HMDB0002048	GC-MS	2	●										
4-Hydroxybenzeneacetic acid	HMDB0000020	GC-MS	2	●		●	●							
3-Hydroxyhippuric acid	Not available	GC-MS	2	●										
D-Lactose	HMDB0041627	GC-MS	1	●			●							
Niacin (Nicotinate/Vitamin B3)	HMDB0001488	GC-MS	1	●	●									
Catechol	HMDB0000957	GC-MS	1	●	●									
Citramalate	HMDB0000426	GC-MS	1			●								
Erythritol	HMDB0002994	GC-MS	1	●		●								
Tartaric acid	HMDB0000956	GC-MS	1	●		●								
3,4-Dihydroxyhydrocinnamic acid	HMDB0000423	GC-MS	2	●										
D-Fucitol[a]	HMDB0304954	GC-MS	2		●									
2,3-Dihydroxybutanoic acid	HMDB0245394	GC-MS	2	●		●	●							
Arabinofuranose	HMDB0012325	GC-MS	2	●		●								
Furoylglycine	HMDB0000439	GC-MS	2	●	●									
2-Keto-l-gluconic acid[a]	HMDB0245186	GC-MS	2	●	●									
Ethyl α-D-glucopyranoside	HMDB0252035	GC-MS	2	●		●	●							
Glyceryl-glycoside ether	Not available	GC-MS	2					●						
Methyluric acid	HMDB0003099	LC-MS	1[b]		●									
Dimethyluric acid	HMDB0001857	LC-MS	1[b]		●									

FB, fermented beverages; FCG, fermented cereals and grains; FD, fermented dairy; GC-MS, gas chromatography mass spectrometry; ID, identification; LC-MS, liquid chromatography mass spectrometry; WG, wholegrain; WT, white.

[a] Based on the Spearman's correlations this metabolite is negatively associated with the fermented foods and food groups indicated, but discriminant based on statistical significance in other tests (*e.g.*, PLS-DA, Random Forest).

[b] Matched on retention time, mass, and fragmentation pattern.

Stability of identified biomarkers with increasing time between biosample collection and food frequency questionnaire completion

Spearman's correlations between the identified FIBs for fermented foods across different times between biosample collection and dietary assessment with the FFQ are presented in **Table S6**. Almost all correlations observed for the identification cohort (FFQ ± 14d) remained significant with increasing time between biosample collection and FFQ completion (FFQ ± 30d, 90d, 180d, and all FFQ), with only slight attenuations when the time between biosample collection and FFQ completion increased. The strongest correlations were observed between self-reported coffee intake and a series of FIBs, including plasma quinate, and urinary niacin, furoylglycine, methyluric acid, and dimethyluric acid ($r_s \geq 0.4$, $p \leq 0.05$). For wine, the strongest correlations included urinary tartaric acid and arabinofuranose ($r_s \sim 0.4$), and for beer, the strongest correlation observed was ethyl α-D-glucopyranoside ($r_s \sim 0.27$) ($p \leq 0.05$). These correlations were also largely echoed between these metabolites and the intake of total fermented beverages. For total fermented cereals/grains, a significant moderate correlation was observed between self-reported intake and urinary glyceryl-glycoside ether in the identification cohort ($r_s \sim 0.37$), but the correlation attenuated in the full metabolomics cohort ($r_s < 0.3$) ($p \leq 0.05$). Conversely, correlations for intakes of cocoa, total fermented dairy, cheese, yoghurt, white bread, and wholegrain bread and their potential FIBs were either weak or non-existent.

Associations between identified biomarkers and cardiometabolic health parameters

In the fully-adjusted model visualized in **Figure S4**, 20 metabolites were positively associated and 10 negatively associated with CMD risk parameters (unadjusted $p \leq 0.05$). After adjusting for multiple comparisons, ten associations remained significant, including between plasma glutamic acid and urinary 2,3-dihydroxypropyl phosphoric acid with BMI (Standardized (Std.) β = 0.28, standard error (SE) = 0.078, R^2 = 0.32; Std. β = 2.22×10^{-7}, SE = 5.37×10^{-8}, R^2 = 0.30, respectively) and waist circumference (Std. β = 1.73×10^{-7}, SE = 4.67×10^{-8}, R^2 = 0.50; Std. β = 0.28, SE = 0.072, R^2 = 0.48, respectively) (all FDR-adjusted $p \leq 0.05$). Additional FDR-adjusted significant associations were observed between plasma xylitol (Std. β = 2.20×10^{-7}, SE = 6.43×10^{-8}, R^2 = 0.26), glutamic acid (Std. β = 0.31, SE = 0.075, R^2 = 0.30), and trigonelline (Std. β = 0.34, SE = 0.10, R^2 = 0.28), as well as urinary niacin (Std. β = 2.55×10^{-7}, SE = 5.66×10^{-8}, R^2 = 0.32), furoylglycine (Std. β = 8.94×10^{-8}, SE = 2.41×10^{-8}, R^2 = 0.29), and methyluric acid (Std. β = 0.34, SE = 0.11, R^2 = 0.28), with SCORE (all FDR-adjusted $p \leq 0.05$). No FDR-adjusted significant associations were observed between metabolites with risk factors in blood (blood lipids, glucose, HbA1c) or blood pressure.

Discussion

FIBs identified for the habitual intake of individual fermented foods

In the current work, we aimed to identify FIBs for fermented foods consumed in the habitual Dutch adult diet, which included coffee, wine, beer, wholegrain bread, white bread, cheese, yoghurt, and cocoa. A total of 12 plasma and 26 urinary metabolites were identified from non-targeted GC-MS and LC-MS analyses, the majority of which corresponded to the intakes of coffee, wine, and beer (no metabolites were identified for cocoa, white bread, wholegrain bread, cheese, and yoghurt intake). These fermented foods were also coincidentally those with the highest intakes (in g/day) in the Dutch adult diet and span a wide range of intakes, which is conducive for the selection of discriminant metabolites. Several of the most promising FIBs identified for these foods were also previously captured by other non-targeted and targeted studies. For instance, plasma/serum quinate and trigonelline, as well as urinary niacin, furoylglycine, catechol, methyluric and dimethyluric acids, have been previously reported as candidate FIBs of habitual coffee intake (57-68). Out of the metabolites identified for wine intake, hydroxy(*iso*)butyric acid has been previously detected in serum after long-term (>4 weeks) wine intake (69), tartaric acid in urine following acute wine intake (70, 71), and urinary

4-hydroxybenzeneacetic acid in urine following both acute and long-term (>4 weeks) wine intake (69, 72-75). The detection of these previously-identified FIBs in our free-living population further supports their status as reliable indicators of the habitual intake of these fermented foods.

Additionally, several metabolites were identified for coffee, wine, and beer intake which have not been previously reported. For instance, we found urinary 3-deoxy-D-ribo-hexonic acid gamma-lactone to be discriminant for coffee intake. This compound is a degradative product of glucose produced during the Maillard reaction (76), which could have formed during coffee brewing. For wine intake, plasma xylitol, plasma/urinary erythritol, and urinary glucuronic acid, citramalate, 2,3-dihydroxybutanoic acid, arabinofuranose and ethyl α-D-glucopyranoside were identified as potential FIBs. Further, for beer intake, plasma L-cysteine, urinary 2,3-dihydroxypropyl phosphoric acid, 4-hydroxybenzeneacetic acid, D-lactose, 2,3-dihydroxybutanoic acid and ethyl α-D-glucopyranoside were identified. While not detected previously in biofluids, almost all of these metabolites have been detected or quantified in the associated foods themselves. Erythritol (a natural sugar alcohol) has been previously detected in multiple fermented foods, including wine, beer, sake, coffee, cheese, and soy sauce (58, 77-79). Interestingly, since erythritol can be produced by microorganisms (*e.g.*, *Penicilllium* sp. used in the ripening of cheese), it could be considered a 'fermentation-dependent' FIB of fermented foods (15, 79). Similarly, citramalate (a microbial metabolite that is found to be a byproduct of *Saccharomyces*, *Propionibacterium acnes,* and *Aspergillus niger*) has been detected in red wine (80, 81). The detection of these metabolites in the plasma and urine metabolomes of free-living individuals consuming these fermented foods indicates that fermentation-dependent metabolites could act as powerful complementary FIBs (in addition to other FIBs originating from the raw food substrate) to improve the accuracy of dietary assessment of fermented foods in future studies. Thus, further validation studies are required in order to confirm the robustness and reliability of these newly-identified FIBs in a separate population.

One major challenge to the validation of single FIBs relates to their non-specificity for a particular food. Indeed, the vast majority of metabolites identified for the intake of coffee, wine, and beer as described above have also been detected in biofluids following the consumption of other foods. For instance, plasma/serum quinic acid and urinary furoylglycine have also been identified for habitual cocoa intake (82, 83), while methyluric and dimethyluric acids, being caffeine metabolites, have naturally also been identified for the intake of caffeinated foods (*i.e.*, cocoa and tea) (83, 84). The phenolic 4-hydroxybenzeneacetic acid corresponding to wine and beer intake has also been detected in urine after acute bread intake (85) and in serum after long-term coffee intake (58). Furthermore, tartaric acid, while a fairly specific FIB for wine intake, has also been identified in urine following acute and long-term bread intake (85) as well as acute beer intake (86). The limitations of using these single metabolites as FIBs could be circumvented by developing reliable multi-marker panels (5). On the other hand, for fermented foods, non-specific markers shared between different foods could also be useful for indicating common raw materials, fermentation processes (*e.g.*, lactic, acetic, alcoholic, or alkaline fermentation), and/or fermentation with common microorganisms (*e.g.*, lactic acid bacteria, with yeast).

FIBs identified for the intake of groups of fermented foods

In the current work we also explored using dry matter as a novel method to unify individual fermented foods with similar qualities into fermented food groups. Several groups were generated: total fermented beverages (comprising coffee, wine, and beer), total fermented cereals/grains (wholegrain and white bread), and total fermented dairy (cheese and yoghurt). By far, the largest number of identified metabolites were discriminant for the intake of total fermented beverages (which comprised coffee, wine, and beer); however, the significance of the majority of these metabolites appeared to be largely driven by the individual beverages under this group. A few metabolites (plasma 2-hydroxybutyric acid, trans-aconitic acid, L-phenylalanine and

isoleucine; urinary guaiacol, 3-hydroxyhippuric acid, and 3,4-dihydroxyhydrocinnamic acid) appeared to be uniquely discriminant for total fermented beverages. One metabolite was identified for total fermented cereals/grains intake (urinary glyceryl-glycoside ether). This metabolite has not been identified as a FIB previously and needs to be validated in further studies. No metabolites were identified for the intakes of cocoa or total fermented dairy, which could be due to the low or inconsistent intake of these foods (in the case of cocoa), or the discriminant metabolites being also of endogenous origin and thus influenced more heavily by human metabolism (in the case of fermented dairy).

While this is the first study to identify FIBs in the context of fermented food groups, this is also an area in need of further development. We formed the fermented food groups based on the groups for which the FFQ was previously validated for (15, 21). Evidently, there could be other strategies to group fermented foods, which could reveal different sets of FIBs. For instance, fermented foods could be grouped based on a common fermentation process (*e.g.*, lactic fermented foods, yeast fermented foods), which may further reveal fermentation-dependent FIBs. Unfortunately, we did not have access to information on the fermenting microorganisms in order to group fermented foods according to this strategy. In addition, while we did not consider a total fermented food group in the current study, a total intake group might be worth exploring in future which would be highly relevant to examine the health impacts of a dietary pattern of fermented foods.

Methodological considerations for the identification and stability of the biomarkers

Although the primary aim of this study was to identify FIBs for the habitual intake of fermented foods, our work also contributes several methodological insights. Firstly, to comprehensively capture the metabolome for FIB identification, we analysed two biosamples (plasma and urine) using two analytical platforms (GC-MS and LC-MS). We anticipated that the 24-h urine samples would better capture FIBs than plasma collected under fasting conditions, as depending on the speed of metabolism, the metabolite may not be detected even several hours after ingestion in plasma. Indeed, a larger number of urinary metabolites were significant and identified. Still, the number of significant urinary metabolites likely represented a smaller fraction of the total significant (and biologically relevant) metabolites in urine, but which are present at relatively low concentrations due to dilution (a necessary step to ensure metabolites are measured within the linear range of the machine). In addition, there could be differences in the metabolism of different metabolites that influence the detection of potential FIBs (*i.e.*, not all metabolites are eliminated in urine). A combination of these factors may explain why only one identified metabolite (erythritol for wine intake) overlapped between plasma and urine.

We also exploited a combination of univariate and multivariate statistical tests to identify the most discriminant FIBs – a strategy that has been explored by an increasing number of groups (87-89). Our efforts reveal that while the results of univariate and multivariate analyses are not always congruent, one statistical test could be useful as a 'validation' of another test for prioritizing metabolites for identification. However, it could be equally interesting (under ideal circumstances) to identify metabolites from both univariate and multivariate tests, to generate complementary sets of FIBs.

Additionally, we investigated the stability of the identified FIBs with increasing timeframes between biosample collection and completion of self-reported dietary assessment. This was a necessary analysis, since the biosample collection did not occur at the same time as the dietary assessment, and could be a source of variability. Amazingly, we observed excellent stability (correlation coefficient and significance was maintained) with increasing time from biosample collection to the FFQ completion (within 14d, 30d, 180d, and all) for almost all of the identified FIBs. The driving force for this stability could the larger numbers of participants in longer timeframes (affording more statistical power). Moreover, these results could indicate that the FFQ used to collect information on self-reported fermented food intake in this study is fairly robust and/or that the diets of this population are very stable.

Associations between identified FIBs and CMD risk parameters

We further examined associations between the identified FIBs with several CMD risk factors as a preliminary analysis to unravel the complex relationships between fermented food consumption and cardiometabolic health. Of the 38 metabolites identified, 26 were significantly associated with CMD risk factors. However, positive associations for 7 metabolites remained after adjustment for multiple comparisons. Moreover, all associations were weak, which may be attributed to the relatively healthy study population that may not have provided the gradient of CMD risk required to observe a large effect size. Thus, these associations need to be confirmed in larger, prospective cohorts or populations with a more distinctive divide between low and high CMD risk. Nonetheless, some of the associations we found have been reported in the literature. For instance, plasma glutamic acid has been positively associated with obesity, particularly with metabolically unhealthy obese phenotypes (90). Other associations are more contested. In some studies, the consumption of non-nutritive sweeteners (which includes xylitol) has been shown to increase weight and waist circumference, as well as the incidence of obesity, hypertension, metabolic syndrome, type II diabetes and cardiovascular events (91). However, several recent systematic reviews have also revealed that use of non-nutritive sweeteners (instead of sugar) reduce energy intake as well as body weight (92, 93). In all studies, the distinct effects of xylitol (compared to other non-nutritive sweeteners) as well as the underlying mechanisms behind these associations have yet to be verified. Similarly, while we observed significant positive associations between urinary niacin and plasma trigonelline with overall CMD risk (SCORE), this is not in line with the literature for specific CMD risk factors. Niacin is used as pharmacotherapy to prevent cardiovascular disease via lowering cholesterol levels in blood (94). In mechanistic studies, trigonelline has been shown to improve insulin sensitivity by interfering with NADPH oxidase gene expression of pathways and mitochondrial electron chain transport (95). Given these conflicting findings, further studies in larger, prospective cohorts are needed to clarify these associations and examine whether they play an intermediate role in CMD.

Conclusions

Through non-targeted metabolomics, we identified 36 metabolites that were discriminant for the intake of several fermented beverages (coffee, wine, and beer), some of which could be promising FIBs for the habitual intake of these foods. Further studies are required to expand the identification of FIBs for other fermented foods (bread, cocoa, cheese, yoghurt), validate the identified FIBs, and further understand the associations between fermented food intake and CMD risk.

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List of abbreviations

BMI, body mass index; CMD, cardiometabolic disease; FDR, false discovery rate; FFQ, food frequency questionnaire; FIB, food intake biomarker; GC-MS, gas chromatography-mass spectrometry; HDL, high-density lipoprotein; liquid chromatography mass spectrometry (LC-MS); LDL, low-density lipoprotein; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; MSI, Metabolomics Standards Initiative; NDARD, National Dietary Assessment Reference Database; NQplus, Nutritional Questionnaire plus; PCA, principal component analysis; PLS-DA, partial least-square discriminant analysis; PUFA, polyunsaturated fatty acid; QC, quality control; SCORE; Systematic COronary Risk Evaluation; VIP, variable importance in projection.

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Supplementary Materials

Table S1. Classification of fermented foods from the NQplus food frequency questionnaire		
Food item for classification	**Fermented food group**	**Fermented food**
Beer	Beverages	Beer
Low-alcohol Beer	Beverages	Beer
Coffee	Beverages	Coffee
Wine, other	Beverages	Wine
Wine, red	Beverages	Wine
Brown bread	Cereals/grains	Bread, brown
Brown bread (slices)	Cereals/grains	Bread, brown
Rye bread	Cereals/grains	Bread, rye
Biscuit (bread), white	Cereals/grains	Bread, white
Bread with raisins	Cereals/grains	Bread, white
White bread	Cereals/grains	Bread, white
White bread (slices)	Cereals/grains	Bread, white
Biscuit (bread), whole wheat	Cereals/grains	Bread, wholegrain
Multiple grain bread	Cereals/grains	Bread, wholegrain
Multiple grain bread (slices)	Cereals/grains	Bread, wholegrain
Whole wheat bread (slices)	Cereals/grains	Bread, wholegrain
Cake / pastry	Cereals/grains	Pastry
Croissants	Cereals/grains	Pastry
Chocolate spread	Cocoa-based products	Chocolate
Milk chocolate	Cocoa-based products	Chocolate
Pure chocolate	Cocoa-based products	Chocolate
White chocolate	Cocoa-based products	Chocolate
Buttermilk	Dairy	Buttermilk
Low-fat cheese (20^{+}/30^{+})	Dairy	Cheese
Regular cheese (40^{+})	Dairy	Cheese
Regular cheese (48^{+})	Dairy	Cheese
Cheese as snack	Dairy	Cheese
Cheese with hot meal	Dairy	Cheese
Fat luxury cheese	Dairy	Cheese
Less-fat luxury cheese	Dairy	Cheese
Unknown cheese	Dairy	Cheese
(Fruit) quark with breakfast	Dairy	Quark
Full (fruit) yogurt	Dairy	Yogurt
Full yogurt	Dairy	Yogurt
Semi-skim (fruit) yogurt	Dairy	Yogurt
Semi-skim yogurt	Dairy	Yogurt
Skim (fruit) yogurt	Dairy	Yogurt
Skim yogurt	Dairy	Yogurt
Unknown yogurt	Dairy	Yogurt

NA, not applicable.

Table S2. Summary of the significant metabolites across univariate and multivariate statistical analyses for selection of the most discriminant compounds[a]

				Spearman's correlation		Kruskal-Wallis		Random Forest				Number
Platform	Biosample	Fermented fooda	Internal identifier	r	FDR-adjuted *p*-value	(FDR-adjusted *p*-value)	PLS-DA (VIP score)	Variable Importance	OBB	Accuracy	*p*-value	of sig. tests
GC-MS	Plasma	Beer	Compound_43	0.238	0.045	NA	2.961	NA	NA	NA	NA	2
GC-MS	Plasma	Beer (dry)	Compound_43	0.238	0.044	NA	2.961	NA	NA	NA	NA	2
GC-MS	Plasma	Coffee	Compound_210	0.427	0.000	0.000	5.786	0.020	0.518	0.491	0.018	4
GC-MS	Plasma	Coffee (dry)	Compound_210	0.427	0.000	0.000	5.786	0.020	0.518	0.491	0.018	4
GC-MS	Plasma	Total FB	Compound_210	0.438	0.000	0.000	5.075	0.024	0.500	0.509	0.009	4
GC-MS	Plasma	Total FB	Compound_2651	0.322	0.003	NA	1.369	NA	NA	NA	NA	2
GC-MS	Plasma	Total FB	Compound_55	NA	NA	NA	2.083	0.003	0.500	0.509	0.009	2
GC-MS	Plasma	Total FB (dry)	Compound_171	0.253	0.031	NA	3.933	NA	NA	NA	NA	2
GC-MS	Plasma	Total FB (dry)	Compound_210	0.259	0.027	0.036	3.366	NA	NA	NA	NA	3
GC-MS	Plasma	Total FB (dry)	Compound_31	0.283	0.008	0.012	3.683	NA	NA	NA	NA	3
GC-MS	Plasma	Total FB (dry)	Compound_43	0.278	0.010	0.036	4.234	NA	NA	NA	NA	3
GC-MS	Plasma	Total FB (dry)	Compound_96	0.352	0.000	0.001	4.303	NA	NA	NA	NA	3
GC-MS	Plasma	White bread (dry)	Compound_48	NA	NA	NA	1.778	0.001	0.589	0.491	0.018	2
GC-MS	Plasma	Wine	Compound_21	0.344	0.000	NA	3.280	NA	NA	NA	NA	2
GC-MS	Plasma	Wine	Compound_96	0.319	0.002	NA	3.883	NA	NA	NA	NA	2
GC-MS	Plasma	Wine (dry)	Compound_21	0.338	0.001	NA	3.072	NA	NA	NA	NA	2
GC-MS	Plasma	Wine (dry)	Compound_96	0.315	0.003	0.018	3.732	NA	NA	NA	NA	3
GC-MS	Urine	Beer	Compound_146	0.232	0.028	0.025	1.842	NA	NA	NA	NA	3
GC-MS	Urine	Beer	Compound_23	NA	NA	0.009	2.117	NA	NA	NA	NA	2
GC-MS	Urine	Beer	Compound_278	0.286	0.003	0.025	2.346	NA	NA	NA	NA	3
GC-MS	Urine	Beer	Compound_4970	0.230	0.028	NA	1.705	NA	NA	NA	NA	2
GC-MS	Urine	Beer	Compound_58	0.215	0.050	0.011	2.046	NA	NA	NA	NA	3
GC-MS	Urine	Beer	Compound_63	NA	NA	0.019	1.707	NA	NA	NA	NA	2
GC-MS	Urine	Beer (dry)	Compound_146	0.232	0.028	0.025	1.842	NA	NA	NA	NA	3
GC-MS	Urine	Beer (dry)	Compound_23	NA	NA	0.009	2.117	NA	NA	NA	NA	2
GC-MS	Urine	Beer (dry)	Compound_278	0.286	0.003	0.025	2.346	NA	NA	NA	NA	3
GC-MS	Urine	Beer (dry)	Compound_4970	0.231	0.028	NA	1.705	NA	NA	NA	NA	2
GC-MS	Urine	Beer (dry)	Compound_58	0.215	0.050	0.011	2.046	NA	NA	NA	NA	3
GC-MS	Urine	Beer (dry)	Compound_63	NA	NA	0.019	1.707	NA	NA	NA	NA	2

Table S2. Summary of the significant metabolites across univariate and multivariate statistical analyses for selection of the most discriminant compounds[a]

				Spearman's correlation		Kruskal-Wallis		Random Forest				Number
Platform	Biosample	Fermented fooda	Internal identifier	r	FDR-adjuted *p*-value	(FDR-adjusted *p*-value)	PLS-DA (VIP score)	Variable Importance	OBB	Accuracy	*p*-value	of sig. tests
GC-MS	Urine	Coffee	Compound_17	0.334	0.000	0.000	1.922	NA	NA	NA	NA	3
GC-MS	Urine	Coffee	Compound_20	0.457	0.000	0.000	2.432	0.005	0.488	0.537	0.002	4
GC-MS	Urine	Coffee	Compound_23	NA	NA	0.028	1.617	NA	NA	NA	NA	2
GC-MS	Urine	Coffee	Compound_2451	0.401	0.000	0.000	2.171	0.004	0.488	0.537	0.002	4
GC-MS	Urine	Coffee	Compound_247	NA	NA	NA	1.145	0.001	0.488	0.537	0.002	2
GC-MS	Urine	Coffee	Compound_29	0.409	0.000	0.000	2.273	0.005	0.488	0.537	0.002	4
GC-MS	Urine	Coffee	Compound_2992	0.280	0.001	0.002	1.502	0.001	0.488	0.537	0.002	4
GC-MS	Urine	Coffee	Compound_3094	NA	NA	0.000	2.100	0.001	0.488	0.537	0.002	3
GC-MS	Urine	Coffee	Compound_56	0.380	0.000	0.000	2.040	0.004	0.488	0.537	0.002	4
GC-MS	Urine	Coffee (dry)	Compound_17	0.334	0.000	0.000	1.922	NA	NA	NA	NA	3
GC-MS	Urine	Coffee (dry)	Compound_20	0.457	0.000	0.000	2.432	0.005	0.488	0.537	0.002	4
GC-MS	Urine	Coffee (dry)	Compound_23	NA	NA	0.028	1.617	NA	NA	NA	NA	2
GC-MS	Urine	Coffee (dry)	Compound_2451	0.401	0.000	0.000	2.171	0.004	0.488	0.537	0.002	4
GC-MS	Urine	Coffee (dry)	Compound_247	NA	NA	NA	1.145	0.001	0.488	0.537	0.002	2
GC-MS	Urine	Coffee (dry)	Compound_29	0.409	0.000	0.000	2.273	0.005	0.488	0.537	0.002	4
GC-MS	Urine	Coffee (dry)	Compound_2992	0.280	0.001	0.002	1.502	0.001	0.488	0.537	0.002	4
GC-MS	Urine	Coffee (dry)	Compound_3094	NA	NA	0.000	2.100	0.001	0.488	0.537	0.002	3
GC-MS	Urine	Coffee (dry)	Compound_56	0.380	0.000	0.000	2.040	0.004	0.488	0.537	0.002	4
GC-MS	Urine	Total FB	Compound_134	NA	NA	0.011	1.402	NA	NA	NA	NA	2
GC-MS	Urine	Total FB	Compound_146	0.293	0.000	0.004	1.731	NA	NA	NA	NA	3
GC-MS	Urine	Total FB	Compound_148	0.203	0.018	NA	1.492	NA	NA	NA	NA	2
GC-MS	Urine	Total FB	Compound_17	0.329	0.000	0.000	1.947	NA	NA	NA	NA	3
GC-MS	Urine	Total FB	Compound_185	0.224	0.007	NA	1.321	NA	NA	NA	NA	2
GC-MS	Urine	Total FB	Compound_20	0.427	0.000	0.000	2.299	NA	NA	NA	NA	3
GC-MS	Urine	Total FB	Compound_23	NA	NA	0.000	2.118	NA	NA	NA	NA	2
GC-MS	Urine	Total FB	Compound_245	0.203	0.018	NA	1.438	NA	NA	NA	NA	2
GC-MS	Urine	Total FB	Compound_2451	0.370	0.000	0.000	2.021	NA	NA	NA	NA	3
GC-MS	Urine	Total FB	Compound_278	0.250	0.002	0.014	2.009	NA	NA	NA	NA	3
GC-MS	Urine	Total FB	Compound_29	0.347	0.000	0.000	2.062	NA	NA	NA	NA	3

Table S2. Summary of the significant metabolites across univariate and multivariate statistical analyses for selection of the most discriminant compounds[a]

				Spearman's correlation		Kruskal-Wallis		Random Forest				Number
Platform	Biosample	Fermented fooda	Internal identifier	r	FDR-adjuted *p*-value	(FDR-adjusted *p*-value)	PLS-DA (VIP score)	Variable Importance	OBB	Accuracy	*p*-value	of sig. tests
GC-MS	Urine	Total FB	Compound_2992	0.269	0.001	0.016	1.312	NA	NA	NA	NA	3
GC-MS	Urine	Total FB	Compound_3094	NA	NA	0.000	1.119	NA	NA	NA	NA	2
GC-MS	Urine	Total FB	Compound_4970	0.247	0.002	NA	1.580	NA	NA	NA	NA	2
GC-MS	Urine	Total FB	Compound_56	0.369	0.000	0.000	2.201	NA	NA	NA	NA	3
GC-MS	Urine	Total FB	Compound_58	0.259	0.001	0.000	1.587	NA	NA	NA	NA	3
GC-MS	Urine	Total FB	Compound_63	0.263	0.001	0.002	1.389	NA	NA	NA	NA	3
GC-MS	Urine	Total FB (dry)	Compound_134	NA	NA	0.016	1.627	0.001	0.401	0.593	0.000	3
GC-MS	Urine	Total FB (dry)	Compound_146	0.378	0.000	0.000	1.958	0.002	0.401	0.593	0.000	4
GC-MS	Urine	Total FB (dry)	Compound_156	0.281	0.001	0.016	1.629	NA	NA	NA	NA	3
GC-MS	Urine	Total FB (dry)	Compound_20	0.259	0.002	NA	1.586	0.001	0.401	0.593	0.000	3
GC-MS	Urine	Total FB (dry)	Compound_23	NA	NA	0.000	2.349	0.006	0.401	0.593	0.000	3
GC-MS	Urine	Total FB (dry)	Compound_278	0.417	0.000	0.000	2.802	0.021	0.401	0.593	0.000	4
GC-MS	Urine	Total FB (dry)	Compound_3874	0.268	0.001	0.004	1.850	0.001	0.401	0.593	0.000	4
GC-MS	Urine	Total FB (dry)	Compound_4970	0.260	0.002	NA	1.695	0.000	0.401	0.593	0.000	3
GC-MS	Urine	Total FB (dry)	Compound_52	NA	NA	0.016	1.665	0.001	0.401	0.593	0.000	3
GC-MS	Urine	Total FB (dry)	Compound_56	0.205	0.019	NA	1.464	NA	NA	NA	NA	2
GC-MS	Urine	Total FB (dry)	Compound_58	0.230	0.007	NA	1.184	NA	NA	NA	NA	2
GC-MS	Urine	Total FB (dry)	Compound_63	0.308	0.000	0.001	2.232	0.001	0.401	0.593	0.000	4
GC-MS	Urine	Total FB (dry)	Compound_96	NA	NA	NA	1.508	0.001	0.401	0.593	0.000	2
GC-MS	Urine	Total FCG	Compound_19	0.383	0.000	0.000	NA	NA	NA	NA	NA	2
GC-MS	Urine	Total FCG (dry)	Compound_19	0.385	0.000	0.000	NA	NA	NA	NA	NA	2
GC-MS	Urine	Wine	Compound_146	0.283	0.001	0.002	2.033	NA	NA	NA	NA	3
GC-MS	Urine	Wine	Compound_156	0.405	0.000	0.000	2.498	NA	NA	NA	NA	3
GC-MS	Urine	Wine	Compound_164	NA	NA	0.044	1.438	NA	NA	NA	NA	2
GC-MS	Urine	Wine	Compound_198	0.235	0.015	NA	1.869	NA	NA	NA	NA	2
GC-MS	Urine	Wine	Compound_278	0.224	0.017	0.040	1.614	NA	NA	NA	NA	3
GC-MS	Urine	Wine	Compound_3874	0.417	0.000	0.000	3.053	NA	NA	NA	NA	3
GC-MS	Urine	Wine	Compound_96	0.338	0.000	0.000	2.665	NA	NA	NA	NA	3
GC-MS	Urine	Wine (dry)	Compound_102	NA	NA	NA	1.639	0.001	0.420	0.463	0.032	2

Table S2. Summary of the significant metabolites across univariate and multivariate statistical analyses for selection of the most discriminant compounds[a]

				Spearman's correlation		Kruskal-Wallis		Random Forest				Number
Platform	Biosample	Fermented fooda	Internal identifier	r	FDR-adjuted *p*-value	(FDR-adjusted *p*-value)	PLS-DA (VIP score)	Variable Importance	OBB	Accuracy	*p*-value	of sig. tests
GC-MS	Urine	Wine (dry)	Compound_146	0.280	0.001	0.002	2.069	0.003	0.420	0.463	0.032	4
GC-MS	Urine	Wine (dry)	Compound_156	0.406	0.000	0.000	2.534	0.006	0.420	0.463	0.032	4
GC-MS	Urine	Wine (dry)	Compound_164	NA	NA	0.036	1.494	0.002	0.420	0.463	0.032	3
GC-MS	Urine	Wine (dry)	Compound_198	0.234	0.016	NA	1.905	NA	NA	NA	NA	2
GC-MS	Urine	Wine (dry)	Compound_278	0.226	0.017	0.036	1.622	0.001	0.420	0.463	0.032	4
GC-MS	Urine	Wine (dry)	Compound_3874	0.415	0.000	0.000	3.066	0.005	0.420	0.463	0.032	4
GC-MS	Urine	Wine (dry)	Compound_96	0.332	0.000	0.000	2.680	NA	NA	NA	NA	3
LC-MS	Plasma	Coffee	Compound_3276	NA	NA	0.034	1.962	0.005	0.532	0.627	0.000	3
LC-MS	Plasma	Coffee	Compound_3903	NA	NA	NA	1.147	0.001	0.532	0.627	0.000	2
LC-MS	Plasma	Coffee (dry)	Compound_3276	NA	NA	0.034	1.962	0.005	0.532	0.627	0.000	3
LC-MS	Plasma	Coffee (dry)	Compound_3903	NA	NA	NA	1.147	0.001	0.532	0.627	0.000	2
LC-MS	Plasma	Wine	Compound_7825	0.597	0.000	0.018	NA	NA	NA	NA	NA	2
LC-MS	Plasma	Wine (dry)	Compound_7825	0.599	0.000	0.018	NA	NA	NA	NA	NA	2
LC-MS	Urine	Coffee	Compound_3885	0.477	0.000	0.000	3.101	0.000	0.400	0.463	0.032	4
LC-MS	Urine	Coffee	Compound_4443	0.496	0.000	0.000	3.082	0.000	0.400	0.463	0.032	4
LC-MS	Urine	Coffee (dry)	Compound_3885	0.477	0.000	0.000	3.101	0.000	0.400	0.463	0.032	4
LC-MS	Urine	Coffee (dry)	Compound_4443	0.496	0.000	0.000	3.082	0.000	0.400	0.463	0.032	4

FB, fermented beverages; FCG, fermented cereals and grains; GC-MS, gas chromatography mass spectrometry; LC-MS, liquid chromatography mass spectrometry; NA, not applicable.

[a] To ensure the length of these data are feasible to include in this thesis, statistical results for only the identified metabolites are included.

Table S3. List of suppliers of analytical standards	
Compound	**Supplier**
Erythritol	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
2-Hydroxybutyric acid	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Cysteine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Dodecanoic acid	Merck, Darmstadt, Germany
Xylitol	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
trans-Aconitic acid	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
D-Quinate	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Phenylalanine	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Isoleucine	Merck, Darmstadt, Germany
D-Psicose	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Glycine	Merck, Darmstadt, Germany
D-Gluconate	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Guaiacol	Sigma-Aldrich, Switzerland
D-Lactose	Merck, Darmstadt, Germany
Niacin	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Catechol	Sigma-Aldrich, Switzerland
Citramalate	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Tartaric acid	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
L-Glutamic acid	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
Trigonelline	MSML kit (IROA Technologies, LLC, Bolton, MA; Gainesville, FL)
(S)-3-Hydroxyisobutyric acid	Sigma-Aldrich, Switzerland, ≥96.0% (sodium salt)
1-Methyluric acid	Toronto Research Chemicals, Toronto, Canada
1,3-Dimethyluric acid	Toronto Research Chemicals, Toronto, Canada

Table S4. Metabolites prioritized for identification from GC-MS (37 plasma, 75 urine)

Internal identifier	Identification	Fermented food(s)	RT (min)	Quantifier Ion	Qualifier Ion	Ratio (Quant/Qual)	RSD QC	RI sample	RI reference	ID level
Plasma[a]										
Compound_0021	Erythritol (4TMS)	Wine, Wine (dry)	25.01	217	205	65	36.6	1491	1494	1
Compound_0031	2-Hydroxybutyric acid (2TMS)	Total FB (dry)	15.72	131	233	7	41.5	1116	1119	1
Compound_0043	L-Cysteine (3TMS)	Beer, Beer (dry), Total FB (dry)	25.96	220	218	90	60.6	1548	1548	1
Compound_0048	Dodecanoic acid (1TMS)	White bread (dry)	27.48	257	117	75	32.3	1647	1649	1
Compound_0096	Xylitol (5TMS)	Total FB (dry), Wine, Wine (dry)	28.2	217	307	35	41.4	1698	1690	1
Compound_0171	trans-Aconitic acid (3TMS)	Total FB (dry)	28.62	375	285	62	46.8	1731	1733	1
Compound_0210	Quinate (5TMS)	Coffee, Coffee (dry), Total FB, Total FB (dry)	29.91	345	255	45	43.1	1839	1843	1
Compound_2651	L-Phenylalanine (1TMS)	Total FB	26.03	120	146	50	61.4	1552	1550	1
Compound_0055	Isoleucine (2TMS)	Total FB	20.5	158	218	22	44.6	1285	1285	1
Compound_0073	Unknown	White bread (dry)	35.74	411	290	350	38.6	2444	n.d.	4
Compound_0094	Unknown	Beer, Beer (dry), Total FB (dry)	35.96	364	290	100	64.4	2467	n.d.	4
Compound_0110	Unknown	Total FB (dry)	31.57	174	n.d.	n.d.	62.9	1992	n.d.	4
Compound_0134	Unknown	White bread (dry)	38.32	290	541	15	79.1	2689	n.d.	4
Compound_0165	Unknown	Beer, Beer (dry), Total FB (dry)	32.31	383	398	65	57.2	2069	n.d.	4
Compound_0173	Unknown	Total FB (dry), Wine, Wine (dry)	30.68	204	n.d.	n.d.	34.2	1907	n.d.	4
Compound_0276	Unknown	White bread (dry)	26.5	172	146	240	52.5	1581	n.d.	4
Compound_0328	Unknown	White bread (dry)	33.14	345	214	50	65.2	2157	n.d.	4
Compound_0611	Unknown	Coffee, Coffee (dry)	26.94	208	192	70	52.0	1609	n.d.	4
Compound_0702	Unknown	White bread (dry)	25.13	337	319	50	33.1	1497	n.d.	4
Compound_0747	Unknown	Coffee, Coffee (dry)	24.06	302	317	75	38.5	1441	n.d.	4
Compound_0823	Unknown	Total FB	27.53	268	181	33	62.6	1651	n.d.	4
Compound_0929	Unknown	Coffee, Coffee (dry)	35	221	n.d.	n.d.	70.4	2364	n.d.	4

Table S4. Metabolites prioritized for identification from GC-MS (37 plasma, 75 urine)										
Internal identifier	**Identification**	**Fermented food(s)**	**RT (min)**	**Quantifier Ion**	**Qualifier Ion**	**Ratio (Quant/Qual)**	**RSD QC**	**RI sample**	**RI reference**	**ID level**
Compound_1088	Unknown	White bread (dry)	26.87	166	255	220	64.1	1604	n.d.	4
Compound_2349	Unknown	Cocoa, Cocoa (dry)	37.82	512	290	87	49.1	2645	n.d.	4
Compound_0104	Unknown	Total FB	35.22	103	232	99	43.1	2390	n.d.	4
Compound_0147	Unknown	Total FB (dry)	31.29	266	237	49	36.6	1967	n.d.	4
Compound_0166	Unknown	Total FB	29.92	297	140	68	50.2	1840	n.d.	4
Compound_0876	Unknown	Wine (dry)	47.27	353	443	28	25.2	3168	n.d.	4
Compound_2273	Unknown	Wine, Wine (dry)	47.27	353	443	28	25.2	3168	n.d.	4
Urine[b]										
Compound_0023	D-Psicose (5TMS 1MEOXa)	Beer, Beer (dry), Coffee, Coffee (dry), Total FB, Total FB (dry)	29.81	307	217	106	24.0	1834	1837	1
Compound_0052	Glycine (3TMS)	Total FB (dry)	20.89	174	248	20	24.2	1300	1300	1
Compound_0164	D-Gluconate (6TMS)	Wine, Wine (dry)	31.42	333	292	90	67.8	1982	1986	1
Compound_0185	Guaiacol (1TMS)	Total FB	18.79	166	181	32	26.6	1226	1224	1
Compound_4970	D-Lactose (8TMS 1MEOXb)	Beer, Beer (dry), Total FB, Total FB (dry)	38.18	361	204	168	24.8	2682	2691	1
Compound_0020	Niacin (1TMS) (Nicotinate/Vitamin B3)	Coffee, Coffee (dry), Total FB, Total FB (dry)	20.78	180	136	50	54.4	1297	1293	1
Compound_0017	Catechol (2TMS)	Coffee, Coffee (dry), Total FB	21.22	254	239	45	23.9	1314	1314	1
Compound_0198	Citramalate (3TMS)	Wine, Wine (dry)	24.44	247	259	20	23.4	1464	1464	1
Compound_0096	Erythritol (4TMS)	Total FB (dry), Wine, Wine (dry)	24.97	217	205	60	21.1	1491	1494	1
Compound_0156	Tartaric acid (4TMS)	Total FB (dry), Wine, Wine (dry)	27.03	292	219	45	21.6	1619	1621	1
Compound_0056	3-deoxy-2,5,6-tris-O-(TMS)-D-ribo-hexonic acid gamma-lactone	Coffee, Coffee (dry), Total FB, Total FB (dry)	29.02	246	129	150	31.2	1767	n.d.	2

Table S4. Metabolites prioritized for identification from GC-MS (37 plasma, 75 urine)

Internal identifier	Identification	Fermented food(s)	RT (min)	Quantifier Ion	Qualifier Ion	Ratio (Quant/Qual)	RSD QC	RI sample	RI reference	ID level
Compound_0058	Phosphoric acid, bis(TMS) 2,3-bis[(trimethylsilyl)oxy]propyl ester (2,3-Dihydroxypropyl phosphoric acid (4TMS))	Beer, Beer (dry), Total FB, Total FB (dry)	28.70	357	445	21	22.2	1742	n.d.	2
Compound_0102	Glucuronic acid (5TMS 1MEOX)	Wine (dry)	30.74	333	160	43	22.1	1917	n.d.	2
Compound_0134	m-Cresol (1TMS)	Total FB, Total FB (dry)	16.67	165	180	43	30.9	1151	n.d.	2
Compound_0146	4-Hydroxybenzeneacetic acid (2TMS)	Beer, Beer (dry), Total FB, Total FB (dry), Wine, Wine (dry)	27.27	252	296	90	25.8	1636	n.d.	2
Compound_0148	3-Hydroxyhippuric acid, O,O'-bis-TMS	Total FB	32.97	294	193	63	28.3	2143	n.d.	2
Compound_0245	3,4-Dihydroxyhydrocinnamic acid (3TMS)	Total FB	30.94	179	267	70	31.6	1936	n.d.	2
Compound_0247	D-Fucitol (5TMS)	Coffee, Coffee (dry)	28.80	117	205	67	34.5	1750	n.d.	2
Compound_0063	2,3-Dihydroxybutanoic acid (3TMS)	Beer, Beer (dry), Total FB, Total FB (dry)	21.87	292	220	40	21.3	n.d.	n.d.	2
Compound_3874	Arabinofuranose (4TMS)	Total FB (dry), Wine, Wine (dry)	26.34	217	218	20	26.1	n.d.	n.d.	2
Compound_0029	Furoylglycine (1TMS)	Coffee, Coffee (dry), Total FB	27.61	95	169	40	36.1	n.d.	n.d.	2
Compound_3094	2-Keto-l-gluconic acid (5TMS)	Coffee, Coffee (dry), Total FB	28.87	292	333	23	30.1	n.d.	n.d.	2
Compound_0278	Ethyl α-D-glucopyranoside (4TMS)	Beer, Beer (dry), Total FB, Total FB (dry), Wine, Wine (dry)	30.17	204	217	22	23.8	n.d.	n.d.	2
Compound_0019	Glyceryl-glycoside TMS ether	Total FCG, Total FCG (dry)	34.18	337	204	555	22.2	n.d.	n.d.	2
Compound_2504	Monosaccharide	Coffee, Coffee (dry), Total FB, Total FB (dry)	30.64	258	332	92	25.2	1908	n.d.	3
Compound_2911	Monosaccharide	Total FB (dry), Wine (dry)	27.94	217	307	95	26.8	1683	n.d.	3

Table S4. Metabolites prioritized for identification from GC-MS (37 plasma, 75 urine)

Internal identifier	Identification	Fermented food(s)	RT (min)	Quantifier Ion	Qualifier Ion	Ratio (Quant/Qual)	RSD QC	RI sample	RI reference	ID level
Compound_4596	Disaccharide	Coffee, Coffee (dry), Total FB	38.09	319	205	42	27.5	2675	n.d.	3
Compound_0158	Sugar alcohol	Total FB (dry), Wine, Wine (dry)	27.99	217	205	35	26.1	n.d.	n.d.	3
Compound_0111	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry)	29.83	267	280	31	20.6	1835	n.d.	4
Compound_2423	Unknown	Total FB (dry), Wine, Wine (dry)	31.20	217	189	9	28.2	1962	n.d.	4
Compound_2451	Unknown	Coffee, Coffee (dry), Total FB	28.19	210	136	61	25.5	1701	n.d.	4
Compound_2494	Unknown	Wine, Wine (dry)	34.88	223	369	38	37.5	2357	n.d.	4
Compound_2606	Unknown	Coffee, Coffee (dry), Total FB	29.84	245	335	11	57.5	1837	n.d.	4
Compound_2621	Unknown	Total FB	31.05	373	358	84	45.9	1947	n.d.	4
Compound_2632	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry)	28.93	292	293	28	31.2	1760	n.d.	4
Compound_2659	Unknown	Total FB (dry)	29.60	217	305	15	30.5	1815	n.d.	4
Compound_2727	Unknown	Beer, Beer (dry), Total FB, Total FB (dry)	30.94	333	292	23	25.2	1937	n.d.	4
Compound_2904	Unknown	Coffee, Coffee (dry), Total FB	29.79	245	246	22	51.5	1832	n.d.	4
Compound_2992	Unknown	Coffee, Coffee (dry), Total FB	29.73	245	335	13	65.4	1826	n.d.	4
Compound_3102	Unknown	Wine, Wine (dry)	35.21	217	103	6.5	25.9	2392	n.d.	4
Compound_3418	Unknown	Wine, Wine (dry)	36.02	388	270	70	47.3	2479	n.d.	4
Compound_3447	Unknown	Wine, Wine (dry)	27.06	288	n.d.	n.d.	22.0	1621	n.d.	4
Compound_3463	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry)	25.39	143	233	65	36.8	1515	n.d.	4
Compound_3497	Unknown	Wine (dry)	26.96	334	232	73	22.7	1614	n.d.	4

Table S4. Metabolites prioritized for identification from GC-MS (37 plasma, 75 urine)

Internal identifier	Identification	Fermented food(s)	RT (min)	Quantifier Ion	Qualifier Ion	Ratio (Quant/Qual)	RSD QC	RI sample	RI reference	ID level
Compound_3503	Unknown	Coffee, Coffee (dry)	30.07	345	346	32	13.1	1857	n.d.	4
Compound_3556	Unknown	Total FB, Total FB (dry)	30.12	297	399	4.3	13.5	1861	n.d.	4
Compound_3729	Unknown	Total FB (dry)	35.25	375	180	76	23.1	2398	n.d.	4
Compound_3860	Unknown	Wine, Wine (dry)	26.33	84	58	85	48.0	1572	n.d.	4
Compound_3887	Unknown	Coffee, Coffee (dry), Total FB	39.35	375	361	96	22.4	2774	n.d.	4
Compound_3942	Unknown	Coffee, Coffee (dry), Total FB	28.14	268	240	6	22.0	1697	n.d.	4
Compound_3947	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry)	35.33	375	376	32	25.1	2406	n.d.	4
Compound_4186	Unknown	Coffee, Coffee (dry)	28.32	294	272	38	27.3	1711	n.d.	4
Compound_4960	Unknown	Wine, Wine (dry)	23.74	239	284	82	27.7	1426	n.d.	4
Compound_4590	Unknown	Total FB (dry)	21.91	245	246	20	26.6	n.d.	n.d.	4
Compound_3083	Unknown	Wine (dry)	25.51	217	307	70	27.5	n.d.	n.d.	4
Compound_2746	Unknown	Coffee, Coffee (dry), Total FB	27.43	245	231	32	37.4	n.d.	n.d.	4
Compound_3015	Unknown	Beer, Beer (dry), Total FB, Total FB (dry), Wine, Wine (dry)	29.37	231	243	20	25.3	n.d.	n.d.	4
Compound_0014	Unknown (possibly quininic acid)	Coffee, Coffee (dry), Total FB, Total FB (dry)	29.88	345	255	35	15.1	n.d.	n.d.	4
Compound_0140	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry)	30.49	298	226	5	22.2	n.d.	n.d.	4
Compound_3712	Unknown (possibly quininic acid)	Coffee, Coffee (dry), Total FB, Total FB (dry)	30.84	345	255	27	23.0	n.d.	n.d.	4
Compound_4400	Unknown	Coffee, Coffee (dry), Total FB,	30.99	389	491	15	33.0	n.d.	n.d.	4

Table S4. Metabolites prioritized for identification from GC-MS (37 plasma, 75 urine)

Internal identifier	Identification	Fermented food(s)	RT (min)	Quantifier Ion	Qualifier Ion	Ratio (Quant/Qual)	RSD QC	RI sample	RI reference	ID level
		Total FB (dry), Wine, Wine (dry)								
Compound_0084	Unknown	Coffee, Coffee (dry), Total FB	31.46	159	217	307	30.2	n.d.	n.d.	4
Compound_4424	Unknown	Coffee, Coffee (dry), Total FB	31.61	429	430	40	60.3	n.d.	n.d.	4
Compound_4289	Unknown	Total FB (dry)	34.63	186	346	26	29.3	n.d.	n.d.	4
Compound_3583	Unknown	Coffee, Coffee (dry), Total FB	35.15	272	375	145	30.5	n.d.	n.d.	4
Compound_4022	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry)	35.68	254	375	240	25.7	n.d.	n.d.	4
Compound_4683	Unknown	Beer, Beer (dry), Coffee, Coffee (dry), Total FB, Total FB (dry)	36.77	375	257	21	27.9	n.d.	n.d.	4
Compound_3973	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry)	37.82	522	507	30	48.2	n.d.	n.d.	4
Compound_2768	Unknown	Beer, Beer (dry), Coffee, Coffee (dry), Total FB, Total FB (dry)	39.19	375	333	10	25.6	n.d.	n.d.	4
Compound_4554	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry), Wine, Wine (dry)	39.24	156	431	18	33.1	n.d.	n.d.	4
Compound_3406	Unknown	Wine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

FB; total fermented beverages; FCG, fermented cereals/grains; FD, fermented dairy; ID, identification; MEOX, methoxyoxamine; n.d., not determined; QC, quality control; RI, retention index; RSD; relative standard deviation; RT, retention time; TMS, trimethylsilyl.

[a]After identification, 8 plasma metabolites were removed due to high levels in blanks or the compounds were found to be originating from the GC column: Compound_0289 (3,5-Diacetyl-4-methyl-1-phenyl-1,4-dihydropyridine), Compound_1281 (Dodecamethylcyclohexasiloxane), Compound_1321 (Decamethylcyclopentasiloxane), Compound_0273 (Alkane), Compound_0083 (Unknown), Compound_2217 (Unknown), Compound_224 (added decane), and Compound_859 (added sucrose).

[b]After identification, 4 urinary metabolites were removed due to high levels in blanks or the compounds were found to be originating from the GC column: Compound_0123 (Dodecamethylpentasiloxane), Compound_0082 (similar to Lumichrome (2MEOX)), Compound_199 (dodecane, 4,6-dimethyl-), and Compound_2723 (unknown).

Table S5. Metabolites prioritized for identification from LC-MS (13 plasma, 89 urine)

Internal identifier	Identification	Food	RT (min)	m/z	Adducts	Measured neutral mass (Da)	ID level
Plasma							
Compound_3903	Glutamic acid	Coffee, Coffee (dry)	0.95	148.0602	M+H	-	1[a]
Compound_3276	Trigonelline	Coffee, Coffee (dry)	1.04	138.0540	M+H	-	1[a]
Compound_7825	Hydroxy(*iso*)butyric acid	Wine, Wine (dry)	1.23	-	M+ACN+Na, 2M+Na, M+ACN+H	104.0483	2
Compound_8569	Unknown	Total FB (dry)	4.63	246.1694	-	-	4
Compound_6870	Unknown	Coffee, Coffee (dry), Total FB, Total FB (dry)	5.61	211.1433	-	-	4
Compound_12051	Unknown	Coffee, Coffee (dry), Total FB	7.19		-	320.1981	4
Compound_17536	Unknown	Coffee, Coffee (dry), Total FB	7.19		-	496.2301	4
Compound_11880	Unknown	Coffee, Coffee (dry)	8.55		-	316.2030	4
Compound_12800	Unknown	Coffee, Coffee (dry)	12.51	339.2495	-	-	4
Compound_275	Unknown	Coffee, Coffee (dry)	1.23	-	-	86.0191	4
Compound_4335	Unknown	Coffee, Coffee (dry)	2.26	156.1380	-	-	4
Compound_5688	Unknown	Coffee, Coffee (dry)	3.81	-	-	92.0623	4
Compound_5857	Unknown	Coffee, Coffee (dry)	4.80	188.1279	-	-	4
Urine							
Compound_3885	Methyluric acid	Coffee, Coffee (dry)	3.08	-	M+H, M+Na, 2M+H, 2M+Na, M+ACN+Na	182.0437	1[b]
Compound_4443	Dimethyluric acid	Coffee, Coffee (dry)	3.58	-	M+H, 2M+H	196.0591	1[b]
Compound_363	Substituted oxazole	Total FB	1.47	108.0806	M+H-H2O	-	3
Compound_4970	Substituted oxazole	Coffee, Coffee (dry), Total FB	2.12	209.1641	M+ACN+H	-	3
Compound_16982	Xanthone	Wine, Wine (dry)	2.57	440.1209	-	-	3
Compound_12011	O-glucuronide	Wine, Wine (dry)	2.8	-	M+H-H2O, M+H	356.0945	3
Compound_9123	Phenylhydrazone derivative	Wine, Wine (dry)	2.85	-	M+H-H2O, M+Na	266.0994	3
Compound_2564	Substituted oxazole	Coffee, Coffee (dry), Total FB	4.1	150.1273	-	-	3
Compound_14478	Phenolic, possibly glucuronidated metabolite of dihydroxyphenylvalerolactone	Wine, Wine (dry)	6.84	385.1130	-	-	3
Compound_10030	Unknown	Total FB (dry), Wine, Wine (dry)	2.83	304.1746	-	-	4

Table S5. Metabolites prioritized for identification from LC-MS (13 plasma, 89 urine)

Internal identifier	Identification	Food	RT (min)	m/z	Adducts	Measured neutral mass (Da)	ID level
Compound_719	Unknown	Total FB	1.58	112.0392	-	-	4
Compound_7380	Unknown	Wine, Wine (dry)	1.87	259.1280	-	-	4
Compound_12196	Unknown	Beer, Beer (dry)	1.87	-	-	324.1196	4
Compound_15147	Unknown	Total FB (dry)	2.05	-	-	417.1472	4
Compound_5924	Unknown	Total FB (dry)	2.07	-	-	208.0940	4
Compound_8761	Unknown	Wine, Wine (dry)	2.07	283.1132	-	-	4
Compound_10249	Unknown	Total FB (dry)	2.07	308.0183	-	-	4
Compound_7960	Unknown	Total FB (dry)	2.08	269.1330	-	-	4
Compound_10198	Unknown	Total FB (dry)	2.08	307.0779	-	-	4
Compound_11886	Unknown	Total FB (dry), Wine, Wine (dry)	2.08	337.0315	-	-	4
Compound_8403	Unknown	Wine, Wine (dry)	2.54	277.0097	-	-	4
Compound_16323	Unknown	Wine, Wine (dry)	2.83	426.1595	-	-	4
Compound_9057	Unknown	Total FB	2.95	-	-	287.0633	4
Compound_13124	Unknown	Coffee, Coffee (dry), Total FB	2.97	-	-	358.0751	4
Compound_6664	Unknown	Beer, Beer (dry), Total FB (dry)	2.99	-	-	245.0716	4
Compound_9769	Unknown	Coffee, Coffee (dry)	3.01	300.0751	-	-	4
Compound_10148	Unknown	Wine, Wine (dry)	3.22	306.1533	-	-	4
Compound_9833	Unknown	Coffee, Coffee (dry), Total FB	3.42	301.1021	-	-	4
Compound_3117	Unknown	Coffee, Coffee (dry), Total FB	3.48	-	-	122.0185	4
Compound_6494	Unknown	Coffee, Coffee (dry), Total FB	3.49	-	-	242.0466	4
Compound_3488	Unknown	Coffee, Coffee (dry)	3.65	174.0908	-	-	4
Compound_11724	Unknown	Wine, Wine (dry)	3.72	-	-	351.0369	4
Compound_12414	Unknown	Total FB (dry), Wine, Wine (dry)	3.81	346.1121	-	-	4
Compound_13751	O-glycosyl compound	Total FB (dry), Wine, Wine (dry)	3.81	-	-	353.1100	4
Compound_12954	Unknown	Wine, Wine (dry)	3.83	356.1529	-	-	4
Compound_9190	Unknown	Wine, Wine (dry)	4.09	-	-	272.1258	4
Compound_9361	Unknown	Wine, Wine (dry)	4.09	293.1659	-	-	4
Compound_12134	Unknown	Total FB	4.1	-	-	323.0458	4
Compound_8207	Unknown	Total FB (dry), Wine, Wine (dry)	4.28	273.1435	-	-	4
Compound_8334	Unknown	Wine, Wine (dry)	4.57	275.9918	-	-	4

Table S5. Metabolites prioritized for identification from LC-MS (13 plasma, 89 urine)

Internal identifier	Identification	Food	RT (min)	m/z	Adducts	Measured neutral mass (Da)	ID level
Compound_13287	Unknown	Wine, Wine (dry)	4.57	-	-	361.0796	4
Compound_10822	Unknown	Total FD	5.53	317.2423	-	-	4
Compound_20505	Unknown	Beer, Beer (dry), Total FB (dry)	5.62	-	-	266.6181	4
Compound_5054	Unknown	Coffee, Coffee (dry), Total FB	5.64	211.1434	-	-	4
Compound_20504	Unknown	Beer, Beer (dry)	5.81	-	-	266.6180	4
Compound_19417	Unknown	Beer, Beer (dry)	6.84	-	-	482.1598	4
Compound_19888	Unknown	Beer, Beer (dry)	7.43	514.2091	-	-	4
Compound_17585	Unknown	Beer, Beer (dry)	3.77	-	-	435.163	4
Compound_20923	Unknown	Beer, Beer (dry)	6.35	-	-	273.6259	4
Compound_12309	Unknown	Total FB	6.42	-	-	326.0993	4
Compound_15487	Unknown	Total FB	6	407.1386	-	-	4
Compound_11010	Unknown	Total FB (dry)	2.07	320.9971	-	-	4
Compound_11641	Unknown	Total FB (dry), Wine, Wine (dry)	3.81	332.1332	-	-	4
Compound_12669	Unknown	Total FB (dry)	3.79	351.0676	-	-	4
Compound_16053	Unknown	Total FB (dry)	2.07	-	-	397.1574	4
Compound_10310	Unknown	Wine, Wine (dry)	4	309.0952	-	-	4
Compound_10609	Unknown	Wine, Wine (dry)	4.92	-	-	331.0717	4
Compound_11901	Unknown	Wine, Wine (dry)	5.83	337.1298	-	-	4
Compound_12013	Unknown	Wine, Wine (dry)	1.98	-	-	356.0948	4
Compound_13285	Unknown	Wine, Wine (dry)	4.71	362.0650	-	-	4
Compound_13753	Unknown	Wine, Wine (dry)	2.97	-	-	370.1373	4
Compound_14065	Unknown	Wine, Wine (dry)	1.73	-	-	376.1217	4
Compound_14148	Unknown	Wine, Wine (dry)	3.72	-	-	396.0329	4
Compound_14466	Unknown	Wine, Wine (dry)	3.69	385.0083	-	-	4
Compound_14671	Unknown	Wine, Wine (dry)	2.99	389.1073	-	-	4
Compound_15246	Unknown	Wine, Wine (dry)	2.8	-	-	384.1258	4
Compound_15247	Unknown	Wine, Wine (dry)	1.98	402.1597	-	-	4
Compound_15433	Unknown	Wine, Wine (dry)	5.46	-	-	388.0997	4
Compound_15479	Unknown	Wine, Wine (dry)	2.81	407.1149	-	-	4
Compound_15481	Unknown	Wine, Wine (dry)	6.21	407.1329	-	-	4

Table S5. Metabolites prioritized for identification from LC-MS (13 plasma, 89 urine)

Internal identifier	Identification	Food	RT (min)	m/z	Adducts	Measured neutral mass (Da)	ID level
Compound_16244	Unknown	Wine, Wine (dry)	6.21	424.1594	-	-	4
Compound_17214	Unknown	Wine, Wine (dry)	1.98	445.1652	-	-	4
Compound_17215	Unknown	Wine, Wine (dry)	2.8	445.1652	-	-	4
Compound_17613	Unknown	Wine, Wine (dry)	2.69	454.1543	-	-	4
Compound_18170	Unknown	Wine, Wine (dry)	3.88	467.1175	-	-	4
Compound_1837	Unknown	Wine, Wine (dry)	6.75	131.0490	-	-	4
Compound_19773	Unknown	Wine, Wine (dry)	3.27	510.1804	-	-	4
Compound_21904	Unknown	Wine, Wine (dry)	5.6	584.1963	-	-	4
Compound_4819	Unknown	Wine, Wine (dry)	6.74	-	-	205.0734	4
Compound_6159	Unknown	Wine, Wine (dry)	2.12	-	-	253.079	4
Compound_7794	Unknown	Wine, Wine (dry)	3.11	266.0686	-	-	4
Compound_7802	Unknown	Wine, Wine (dry)	2.87	266.1048	-	-	4
Compound_7959	Unknown	Wine, Wine (dry)	1.73	269.1327	-	-	4
Compound_8260	Unknown	Wine, Wine (dry)	3.86	274.1178	-	-	4
Compound_9076	Unknown	Wine, Wine (dry)	4.4	288.1796	-	-	4
Compound_9333	Unknown	Wine, Wine (dry)	6.21	293.0317	-	-	4
Compound_10055	Unknown	Wine (dry)	6.75	-	-	303.99	4
Compound_12553	Unknown	Wine (dry)	3.72	349.0702	-	-	4
Compound_14045	Unknown	Wine (dry)	3.67	377.0289	-	-	4

FB; total fermented beverages; FCG, fermented cereals/grains; FD, fermented dairy; ID, identification; NA, not applicable; RT, retention time.

[a] Matched on retention time and mass; information on fragmentation not available.

[b] Matched on retention time, mass, and fragmentation pattern.

Table S6. Spearman's correlations between identified metabolites and self-reported fermented food intakes

Identification	Fermented foods and groups	Platform	Biosample	Spearman's correlations between metabolites and self-reported fermented food intakes													
				FFQ±14d			FFQ±30d			FFQ±90d			FFQ±180d			All FFQ		
				r	*p*-value	FDR adjusted *p*-value	r	*p*-value	FDR adjusted *p*-value	r	*p*-value	FDR adjusted *p*-value	r	*p*-value	FDR adjusted *p*-value	r	*p*-value	FDR adjusted *p*-value
Erythritol	Wine (dry)	GC-MS	Plasma	0.251	***	**	0.220	***	**	0.191	***	**	0.176	***	**	0.181	***	***
	Wine	GC-MS	Plasma	0.254	***	**	0.221	***	**	0.192	***	**	0.174	***	**	0.180	***	***
Quinate	Coffee (dry)	GC-MS	Plasma	0.441	***	***	0.443	***	***	0.424	***	***	0.409	***	***	0.401	***	***
	Coffee	GC-MS	Plasma	0.441	***	***	0.443	***	***	0.424	***	***	0.409	***	***	0.401	***	***
	Total FB (dry)	GC-MS	Plasma	0.288	***	***	0.271	***	***	0.227	***	***	0.214	***	***	0.207	***	***
	Total FB	GC-MS	Plasma	0.446	***	***	0.444	***	***	0.401	***	***	0.392	***	***	0.381	***	***
L-Phenylalanine	Total FB	GC-MS	Plasma	0.156	*	*	0.149	*	*	0.133	*	*	0.112	*	*	0.109	*	*
2-Hydroxybutyric acid	Total FB (dry)	GC-MS	Plasma	0.384	***	***	0.317	***	***	0.290	***	***	0.219	***	***	0.229	***	***
L-Cysteine	Beer (dry)	GC-MS	Plasma	0.210	**	**	0.172	**	*	0.173	**	**	0.144	**	**	0.146	**	**
	Beer	GC-MS	Plasma	0.210	**	**	0.172	**	*	0.173	**	**	0.144	**	**	0.146	**	**
	Total FB (dry)	GC-MS	Plasma	0.303	***	***	0.226	***	*	0.226	***	***	0.186	***	***	0.173	***	***
Dodecanoic acid [a]	White bread (dry)	GC-MS	Plasma	-0.128	*	0.438	-0.108	0.077	0.488	-0.085	0.118	0.745	-0.023	0.597	0.654	-0.027	0.533	0.699
Isoleucine	Total FB	GC-MS	Plasma	0.200	**	**	0.190	**	**	0.174	**	**	0.134	**	**	0.139	**	**
Xylitol	Total FB (dry)	GC-MS	Plasma	0.394	***	***	0.317	***	***	0.317	***	***	0.230	***	***	0.230	***	***
	Wine (dry)	GC-MS	Plasma	0.275	***	***	0.222	***	**	0.206	***	**	0.137	**	**	0.135	**	**
	Wine	GC-MS	Plasma	0.275	***	***	0.221	***	**	0.204	***	**	0.136	**	**	0.135	**	**
Glucuronic acid [a]	Wine (dry)	GC-MS	Urine	-0.163	*	*	-0.198	**	**	-0.235	***	***	-0.261	***	***	-0.268	***	***
m-Cresol [a]	Total FB	GC-MS	Urine	-0.185	**	**	-0.185	**	**	-0.175	**	**	-0.130	**	**	-0.133	**	**
	Total FB (dry)	GC-MS	Urine	-0.215	**	**	-0.176	**	**	-0.173	**	**	-0.120	**	**	-0.129	**	**
4-Hydroxybenzeneacetic acid	Total FB	GC-MS	Urine	0.254	***	***	0.279	***	***	0.264	***	***	0.215	***	***	0.219	***	***
	Total FB (dry)	GC-MS	Urine	0.334	***	***	0.330	***	***	0.323	***	***	0.249	***	***	0.256	***	***
	Wine	GC-MS	Urine	0.271	***	***	0.251	***	***	0.228	***	***	0.190	***	***	0.197	***	***
	Wine (dry)	GC-MS	Urine	0.266	***	***	0.246	***	***	0.225	***	***	0.189	***	***	0.196	***	***
	Beer	GC-MS	Urine	0.185	**	*	0.170	**	*	0.184	**	**	0.126	**	*	0.128	**	*
	Beer (dry)	GC-MS	Urine	0.185	**	*	0.170	**	*	0.184	**	**	0.126	**	*	0.128	**	*
3-Hydroxyhippuric acid	Total FB	GC-MS	Urine	0.214	**	**	0.267	***	***	0.299	***	***	0.336	***	***	0.333	***	***
Tartaric acid	Total FB (dry)	GC-MS	Urine	0.257	***	***	0.251	***	***	0.275	***	***	0.260	***	***	0.280	***	***
	Wine	GC-MS	Urine	0.388	***	***	0.353	***	***	0.384	***	***	0.398	***	***	0.417	***	***
	Wine (dry)	GC-MS	Urine	0.387	***	***	0.352	***	***	0.385	***	***	0.396	***	***	0.416	***	***
D-Gluconate	Wine	GC-MS	Urine	0.186	**	*	0.165	**	*	0.111	*	0.056	0.105	*	*	0.109	*	*
	Wine (dry)	GC-MS	Urine	0.185	**	*	0.164	**	*	0.111	*	0.057	0.106	*	*	0.110	**	*

Catechol	Total FB	GC-MS	Urine	0.322	***	***	0.345	***	***	0.342	***	***	0.294	***	***	0.285	***	***
	Coffee	GC-MS	Urine	0.327	***	***	0.350	***	***	0.329	***	***	0.293	***	***	0.287	***	***
	Coffee (dry)	GC-MS	Urine	0.327	***	***	0.350	***	***	0.329	***	***	0.293	***	***	0.287	***	***
Guaiacol	Total FB	GC-MS	Urine	0.234	***	**	0.227	***	***	0.229	***	***	0.217	***	***	0.222	***	***
Glyceryl-glycoside TMS ether	Total FCG	GC-MS	Urine	0.365	***	***	0.367	***	***	0.311	***	***	0.291	***	***	0.289	***	***
	Total FCG (dry)	GC-MS	Urine	0.365	***	***	0.366	***	***	0.311	***	***	0.296	***	***	0.295	***	***
Citramalate	Wine	GC-MS	Urine	0.249	***	**	0.215	***	**	0.259	***	***	0.242	***	***	0.247	***	***
	Wine (dry)	GC-MS	Urine	0.247	***	**	0.214	***	**	0.258	***	***	0.244	***	***	0.248	***	***
Niacin (Nicotinate/Vitamin B3)	Total FB	GC-MS	Urine	0.421	***	***	0.401	***	***	0.341	***	***	0.282	***	***	0.275	***	***
	Total FB (dry)	GC-MS	Urine	0.244	***	***	0.256	***	***	0.232	***	***	0.172	***	***	0.176	***	***
	Coffee	GC-MS	Urine	0.462	***	***	0.425	***	***	0.356	***	***	0.306	***	***	0.301	***	***
	Coffee (dry)	GC-MS	Urine	0.462	***	***	0.425	***	***	0.356	***	***	0.306	***	***	0.301	***	***
D-Psicose [a]	Total FB	GC-MS	Urine	-0.368	***	***	-0.382	***	***	-0.341	***	***	-0.307	***	***	-0.312	***	***
	Total FB (dry)	GC-MS	Urine	-0.380	***	***	-0.387	***	***	-0.369	***	***	-0.324	***	***	-0.327	***	***
	Coffee	GC-MS	Urine	-0.227	***	**	-0.258	***	***	-0.232	***	***	-0.208	***	***	-0.217	***	***
	Coffee (dry)	GC-MS	Urine	-0.227	***	**	-0.258	***	***	-0.232	***	***	-0.208	***	***	-0.217	***	***
	Beer	GC-MS	Urine	-0.292	***	***	-0.275	***	***	-0.269	***	***	-0.223	***	***	-0.214	***	***
	Beer (dry)	GC-MS	Urine	-0.292	***	***	-0.275	***	***	-0.269	***	***	-0.222	***	***	-0.214	***	***
3,4-Dihydroxyhydrocinnamic acid	Total FB	GC-MS	Urine	0.210	**	**	0.206	**	**	0.159	**	**	0.152	***	**	0.167	***	***
D-Fucitol [a]	Coffee	GC-MS	Urine	-0.182	**	*	-0.146	*	*	-0.116	*	*	-0.105	*	*	-0.091	*	*
	Coffee (dry)	GC-MS	Urine	-0.182	**	*	-0.146	*	*	-0.116	*	*	-0.105	*	*	-0.091	*	*
Ethyl α-D-glucopyranoside	Total FB	GC-MS	Urine	0.230	***	**	0.199	**	**	0.241	***	***	0.233	***	***	0.252	***	***
	Total FB (dry)	GC-MS	Urine	0.395	***	***	0.389	***	***	0.412	***	***	0.388	***	***	0.394	***	***
	Wine	GC-MS	Urine	0.203	**	**	0.224	***	**	0.273	***	***	0.274	***	***	0.287	***	***
	Wine (dry)	GC-MS	Urine	0.202	**	**	0.223	***	**	0.274	***	***	0.277	***	***	0.290	***	***
	Beer	GC-MS	Urine	0.271	***	***	0.258	***	***	0.253	***	***	0.232	***	***	0.229	***	***
	Beer (dry)	GC-MS	Urine	0.271	***	***	0.258	***	***	0.253	***	***	0.232	***	***	0.229	***	***
Furoylglycine	Total FB	GC-MS	Urine	0.349	***	***	0.351	***	***	0.365	***	***	0.375	***	***	0.378	***	***
	Coffee	GC-MS	Urine	0.421	***	***	0.411	***	***	0.401	***	***	0.423	***	***	0.429	***	***
	Coffee (dry)	GC-MS	Urine	0.421	***	***	0.411	***	***	0.401	***	***	0.423	***	***	0.429	***	***
2-Keto-l-gluconic acid [a]	Total FB	GC-MS	Urine	-0.281	***	***	-0.267	***	***	-0.263	***	***	-0.254	***	***	-0.238	***	***
	Coffee	GC-MS	Urine	-0.316	***	***	-0.301	***	***	-0.292	***	***	-0.263	***	***	-0.254	***	***
	Coffee (dry)	GC-MS	Urine	-0.316	***	***	-0.301	***	***	-0.292	***	***	-0.263	***	***	-0.254	***	***
Arabinofuranose	Total FB (dry)	GC-MS	Urine	0.257	***	***	0.269	***	***	0.272	***	***	0.257	***	***	0.260	***	***
	Wine	GC-MS	Urine	0.400	***	***	0.410	***	***	0.403	***	***	0.398	***	***	0.400	***	***
	Wine (dry)	GC-MS	Urine	0.397	***	***	0.406	***	***	0.397	***	***	0.394	***	***	0.397	***	***

D-Lactose	Total FB	GC-MS	Urine	0.226	***	**	0.233	***	***	0.222	***	***	0.188	***	***	0.200	***	***
	Total FB (dry)	GC-MS	Urine	0.246	***	***	0.262	***	***	0.263	***	***	0.221	***	***	0.232	***	***
	Beer	GC-MS	Urine	0.203	**	**	0.191	**	*	0.182	**	**	0.087	*	0.109	0.083	0.051	0.107
	Beer (dry)	GC-MS	Urine	0.203	**	**	0.191	**	*	0.181	**	**	0.087	*	0.108	0.083	0.051	0.107
Glycine[a]	Total FB	GC-MS	Urine	-0.199	**	**	-0.188	**	**	-0.192	***	**	-0.167	***	***	-0.161	***	***
3-deoxy-D-ribo-hexonic acid gamma-lactone	Total FB	GC-MS	Urine	0.378	***	***	0.386	***	***	0.394	***	***	0.363	***	***	0.356	***	***
	Total FB (dry)	GC-MS	Urine	0.211	**	**	0.229	***	***	0.266	***	***	0.200	***	***	0.205	***	***
	Coffee	GC-MS	Urine	0.394	***	***	0.395	***	***	0.390	***	***	0.387	***	***	0.380	***	***
	Coffee (dry)	GC-MS	Urine	0.394	***	***	0.395	***	***	0.390	***	***	0.387	***	***	0.380	***	***
2,3-Dihydroxypropyl phosphoric acid	Total FB	GC-MS	Urine	0.245	***	***	0.239	***	***	0.237	***	***	0.162	***	***	0.171	***	***
	Total FB (dry)	GC-MS	Urine	0.190	**	**	0.175	**	**	0.203	***	***	0.123	**	**	0.142	**	**
	Beer	GC-MS	Urine	0.210	**	**	0.176	**	*	0.220	***	***	0.139	**	**	0.136	**	**
	Beer (dry)	GC-MS	Urine	0.210	**	**	0.176	**	*	0.220	***	***	0.139	**	**	0.136	**	**
2,3-Dihydroxybutanoic acid	Total FB	GC-MS	Urine	0.241	***	***	0.202	**	**	0.234	***	***	0.142	**	**	0.164	***	***
	Total FB (dry)	GC-MS	Urine	0.261	***	***	0.218	***	**	0.279	***	***	0.206	***	***	0.232	***	***
	Beer	GC-MS	Urine	0.170	**	*	0.176	**	*	0.264	***	***	0.209	***	***	0.214	***	***
	Beer (dry)	GC-MS	Urine	0.170	**	*	0.176	**	*	0.264	***	***	0.209	***	***	0.214	***	***
Erythritol	Total FB (dry)	GC-MS	Urine	0.139	*	*	0.134	*	*	0.201	***	***	0.196	***	***	0.213	***	***
	Wine	GC-MS	Urine	0.315	***	***	0.285	***	***	0.314	***	***	0.312	***	***	0.327	***	***
	Wine (dry)	GC-MS	Urine	0.307	***	***	0.278	***	***	0.309	***	***	0.309	***	***	0.324	***	***
Trigonelline	Coffee	LC-MS	Plasma	0.308	***	***	0.315	***	***	0.303	***	***	0.343	***	***	0.352	***	***
	Coffee (dry)	LC-MS	Plasma	0.308	***	***	0.315	***	***	0.303	***	***	0.343	***	***	0.352	***	***
Glutamic acid	Coffee	LC-MS	Plasma	0.203	**	**	0.190	**	**	0.159	**	**	0.140	**	**	0.145	**	**
	Coffee (dry)	LC-MS	Plasma	0.203	**	**	0.190	**	**	0.159	**	**	0.140	**	**	0.145	**	**
Hydroxy(*iso*)butyric acid	Wine	LC-MS	Plasma	0.357	***	***	0.356	***	***	0.326	***	***	0.263	***	***	0.265	***	***
	Wine (dry)	LC-MS	Plasma	0.360	***	***	0.358	***	***	0.327	***	***	0.263	***	***	0.266	***	***
Methyluric acid	Coffee	LC-MS	Urine	0.490	***	***	0.490	***	***	0.504	***	***	0.491	***	***	0.493	***	***
	Coffee (dry)	LC-MS	Urine	0.490	***	***	0.490	***	***	0.504	***	***	0.491	***	***	0.493	***	***
Dimethyluric acid	Coffee	LC-MS	Urine	0.493	***	***	0.499	***	***	0.518	***	***	0.499	***	***	0.508	***	***
	Coffee (dry)	LC-MS	Urine	0.493	***	***	0.499	***	***	0.518	***	***	0.499	***	***	0.508	***	***

FFQ, food frequency questionnaire; GC-MS, gas chromatography mass spectrometry; LC-MS, liquid chromatography mass spectrometry. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. The strength of the correlation coefficients are visualized along a colour gradient of red (positive correlations) to blue (negative correlations).

[a] Based on the Spearman's correlations this metabolite is negatively associated with the fermented foods and food groups indicated, but discriminant based on statistical significance in other tests (*e.g.*, PLS-DA, Random Forest).

Figure S1. Principal Components Analysis (PCAs) of individual participant samples of the metabolomics subcohort prior to and after removal of outliers. Samples analysed by GC-MS: (a) plasma samples prior to outlier removal and (b) after outlier removal; (c) urine samples prior to outlier removal and (d) after outlier removal. Samples analysed by LC-MS: (e) plasma samples prior to outlier removal and (f) after outlier removal; (g) urine samples prior to outlier removal and (h) after outlier removal.

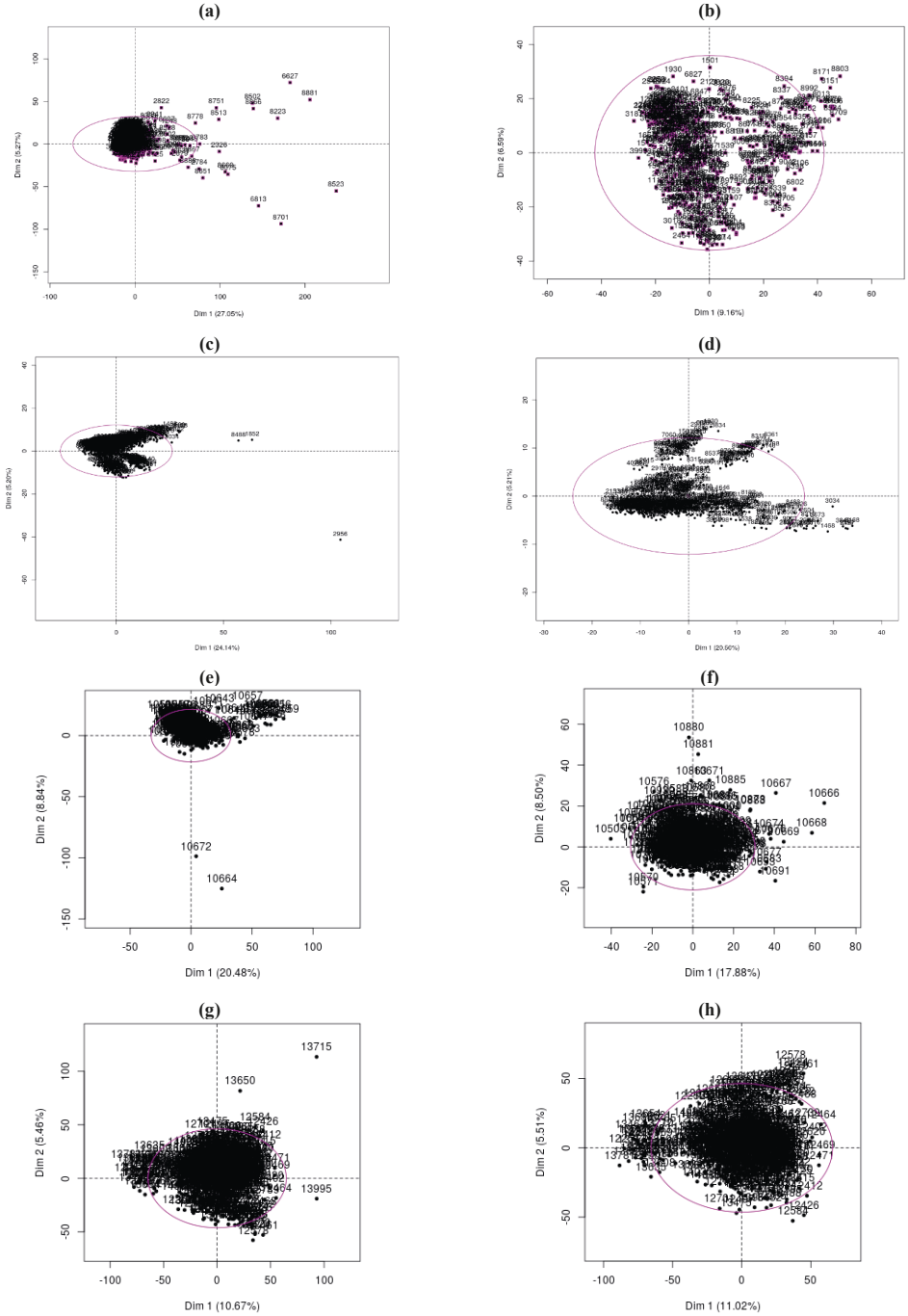


Figure S2. Principal Components Analysis (PCAs) of quality control samples analysed by GC-MS: (a) plasma and (b) urine; LC-MS (c) plasma and (d) urine.

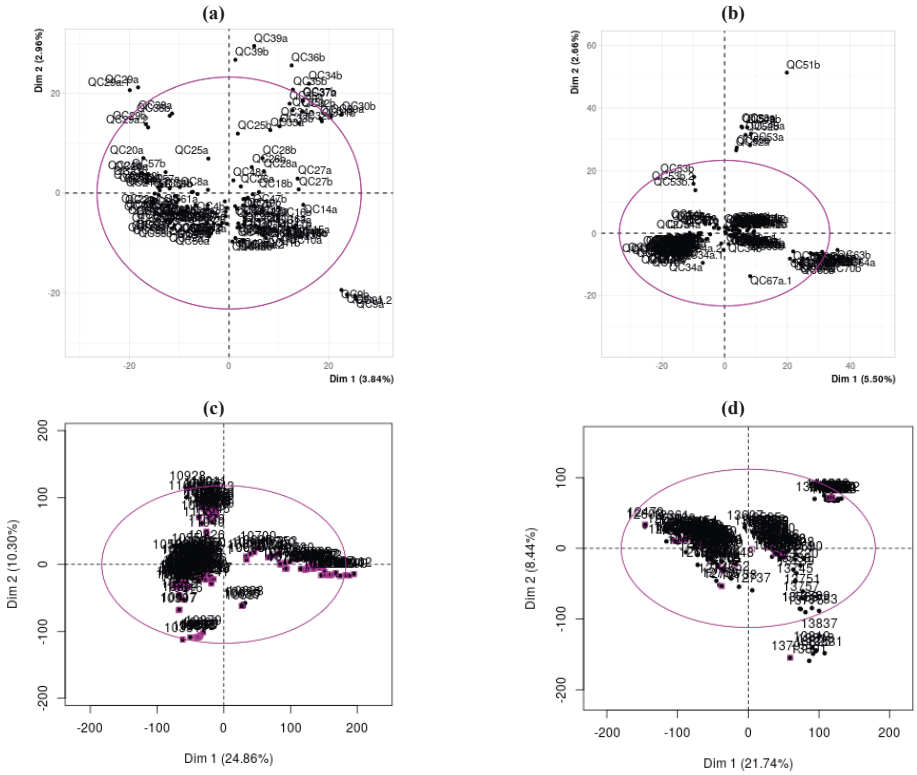
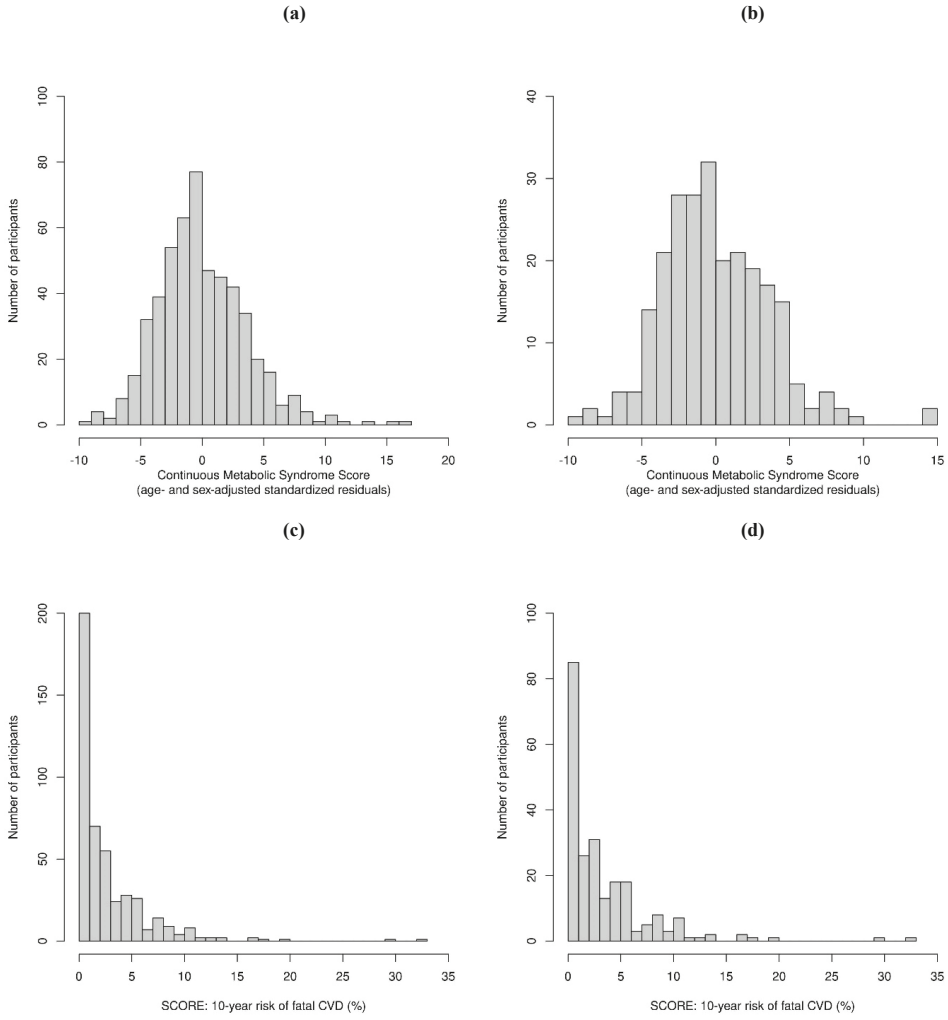


Figure S3. Distribution of (a) continuous metabolic syndrome in (a) the metabolomics subcohort (n = 531) and (b) the identification subcohort (n = 246). Distribution of (c) SCORE in the metabolomics subcohort (n = 531) and (b) the identification subcohort (n = 246).



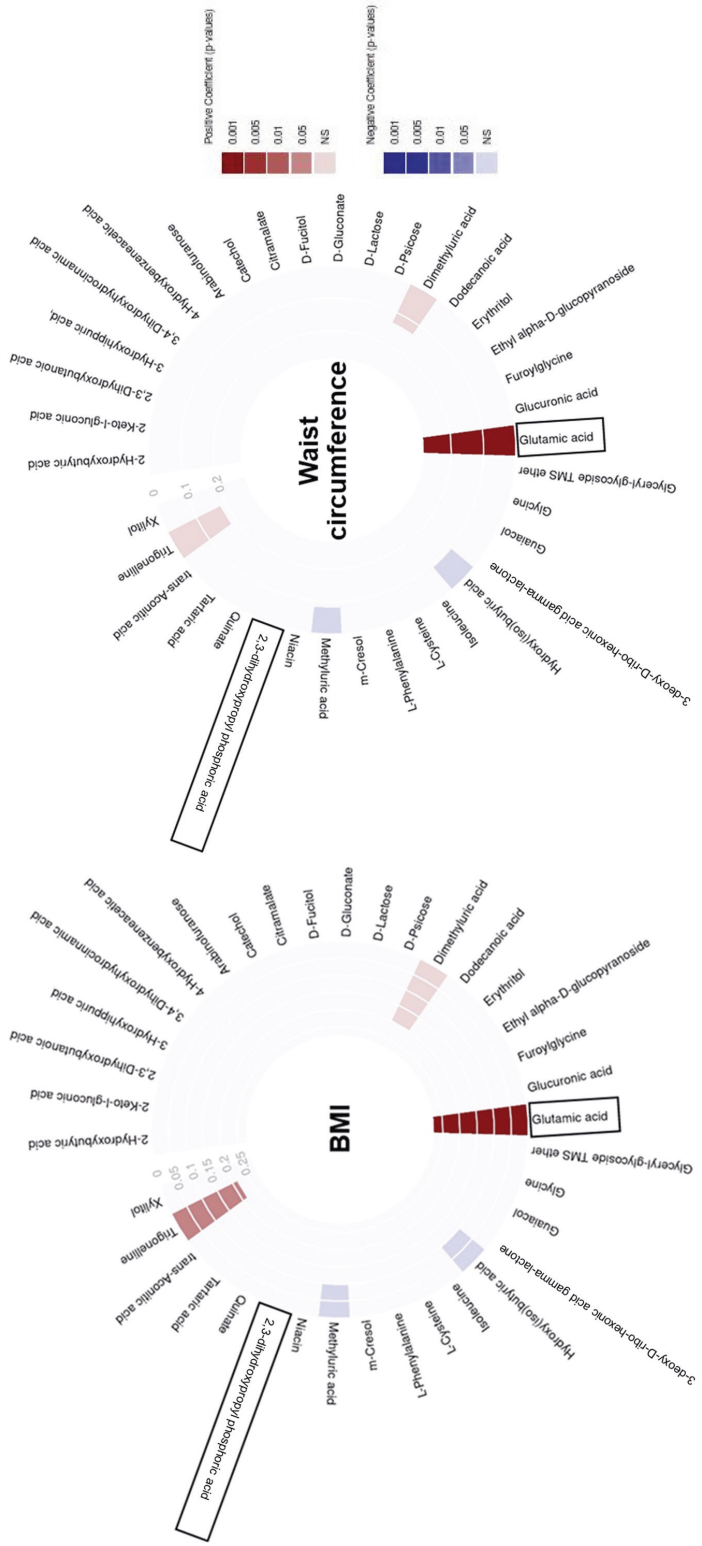


Figure S4. Summary of the associations between identified metabolites and selected CMD risk factors in the fully-adjusted model (Model 3 – adjusted for age, sex, physical activity, education level, smoking, alcohol, vegetables, fruits, meat, and confectionary/desserts intake; for SCORE, adjusted for physical activity, education level, alcohol, vegetables, fruits, meat, and confectionary/desserts intake). The magnitude of the standardized regression coefficients (Std. β) are indicated in each layer of the circle plot, and the direction and significance of the associations are indicated as a colour gradient. Significant FDR-adjusted associations are boxed in black.

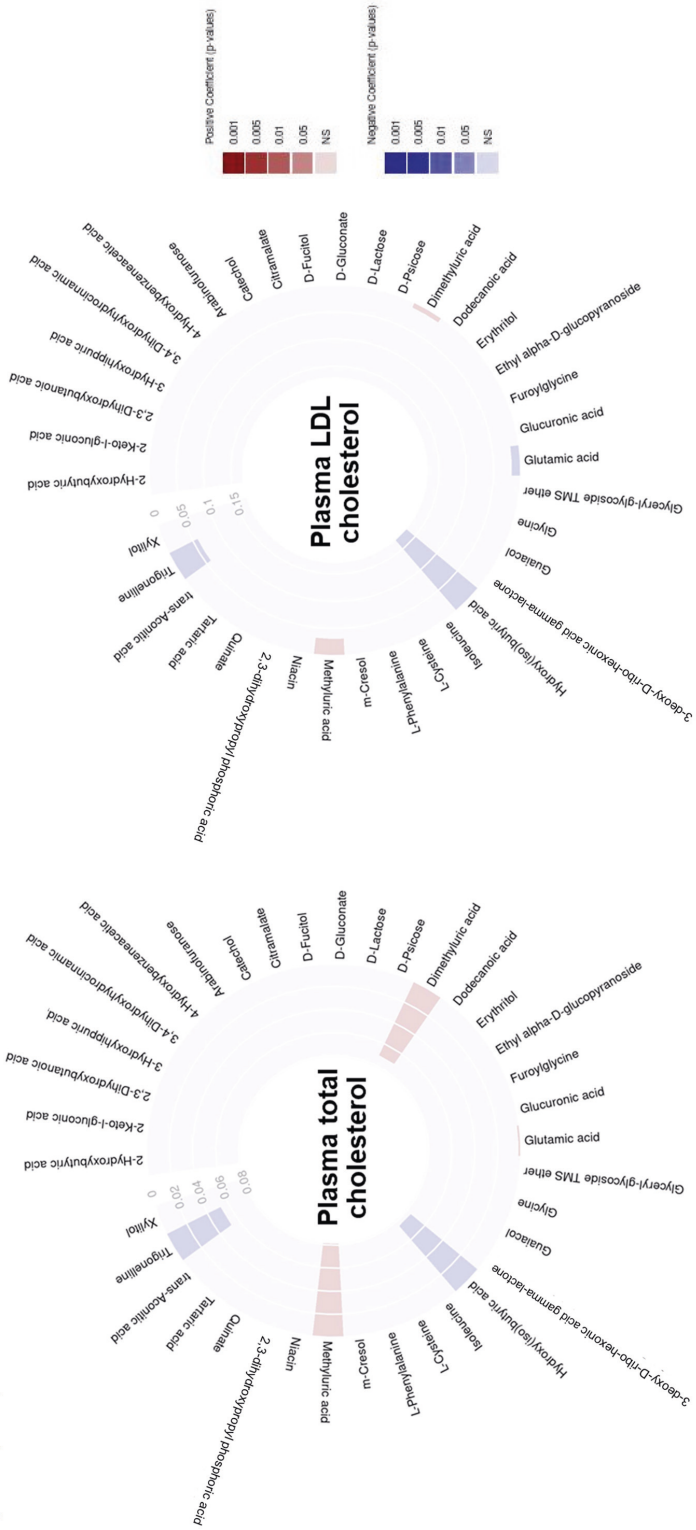
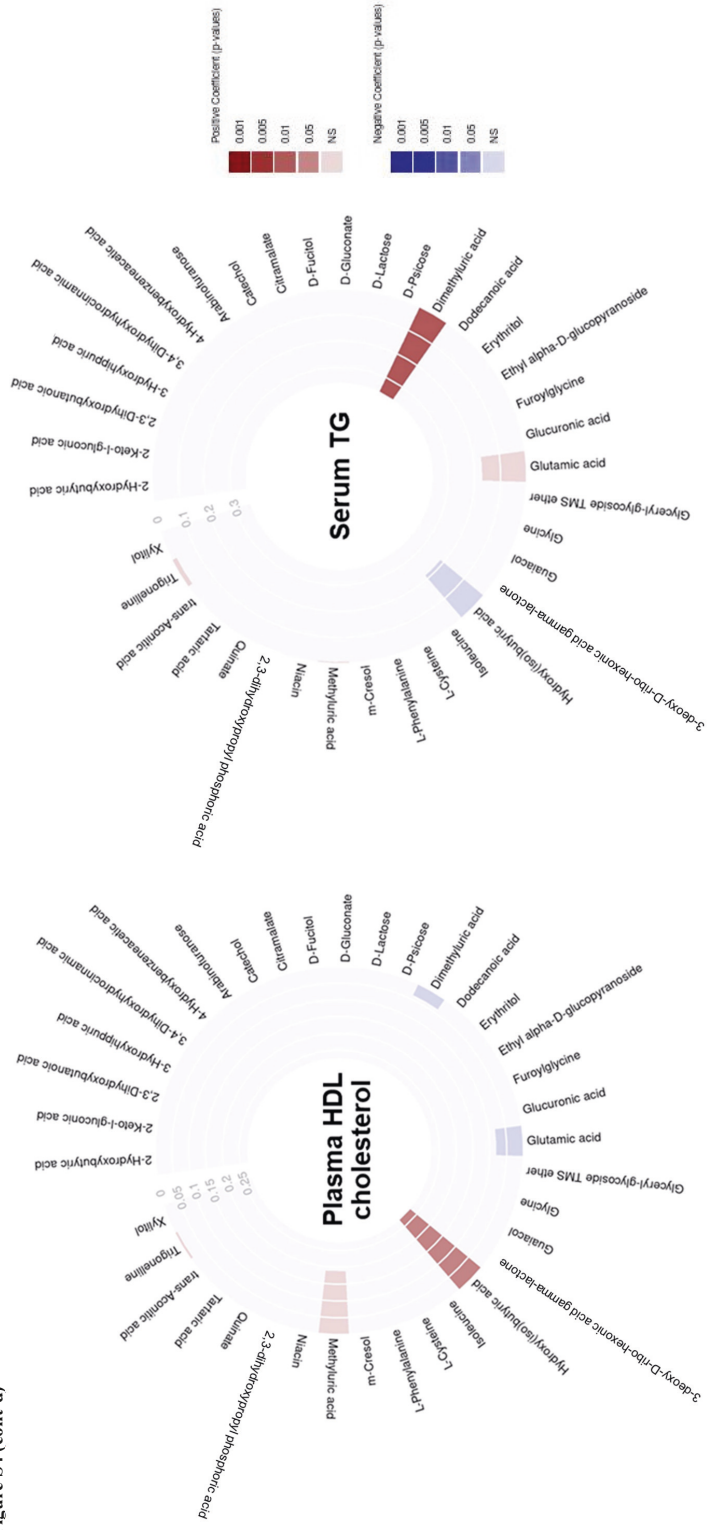


Figure S4 (cont'd)



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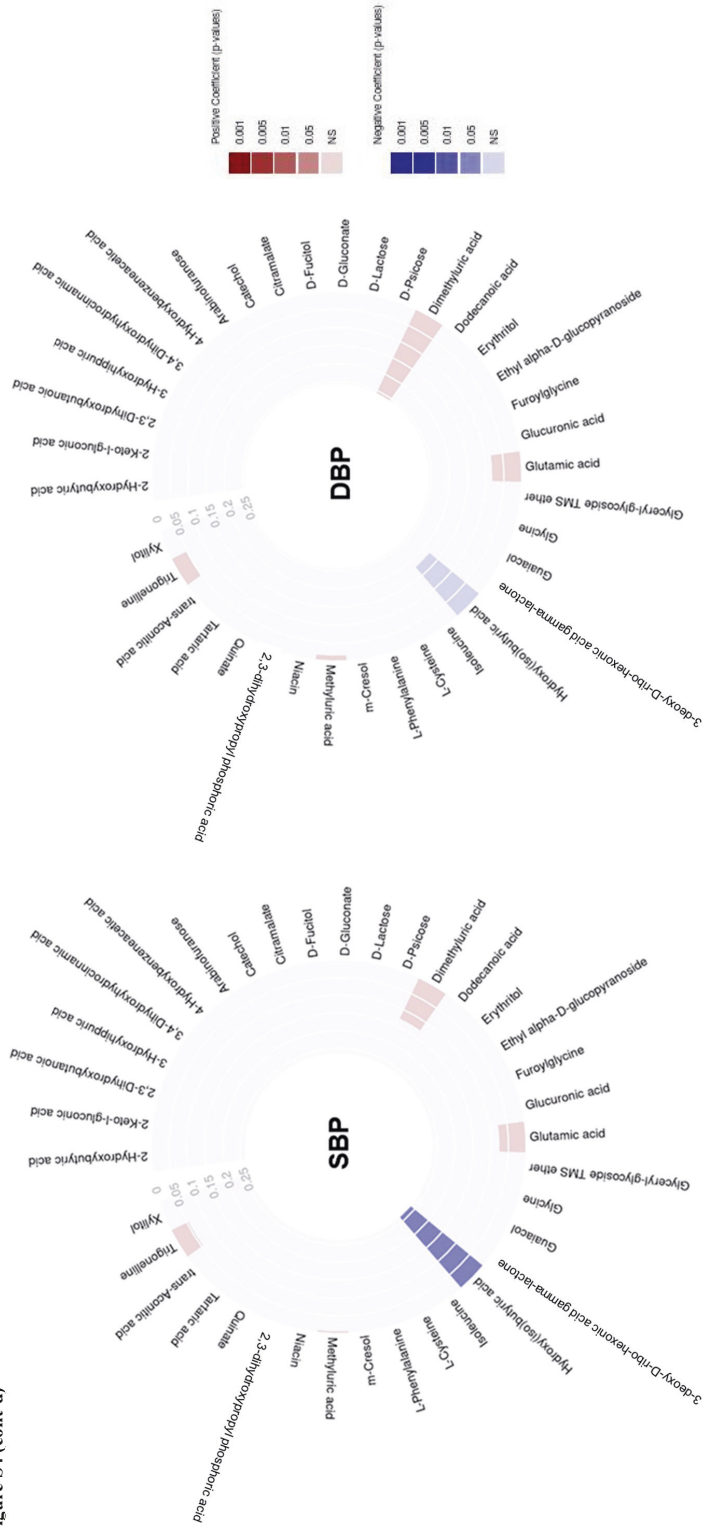

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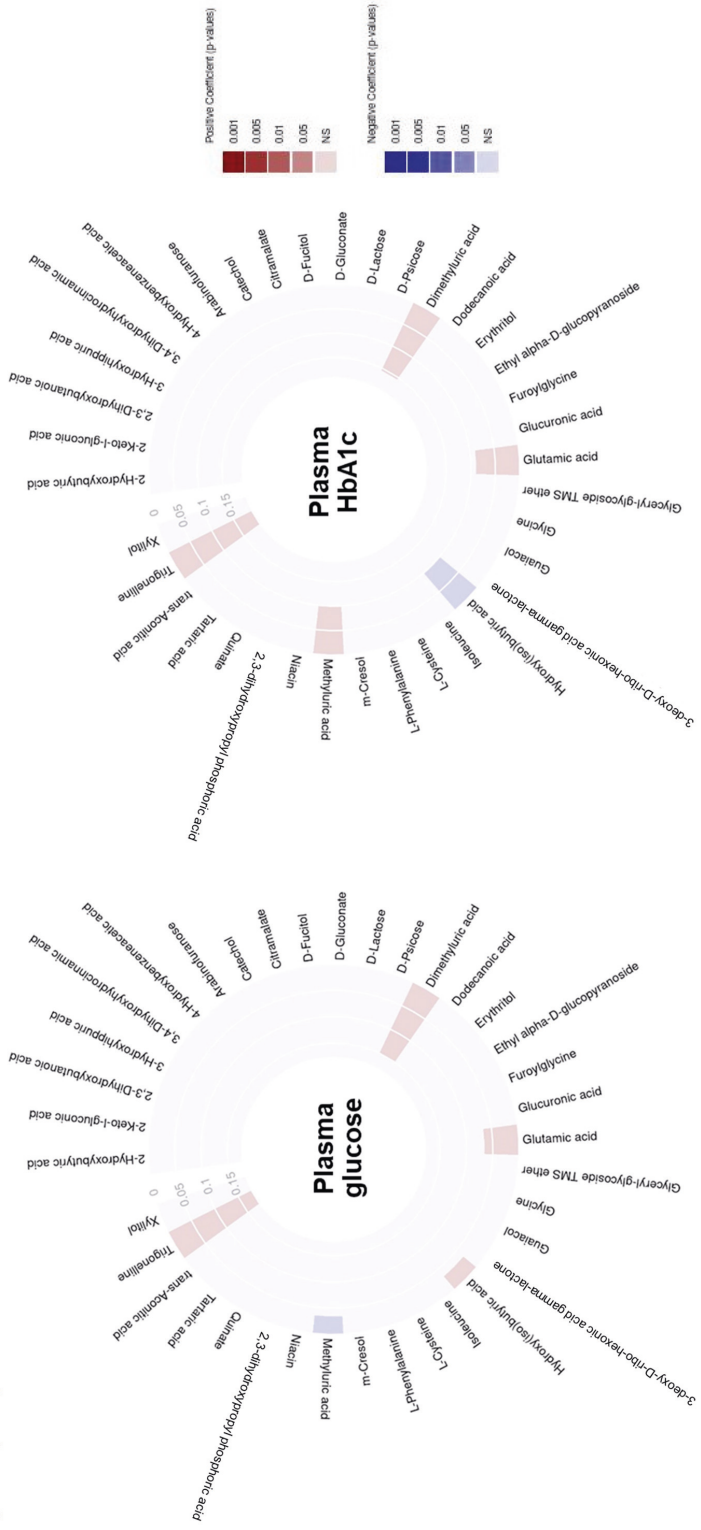

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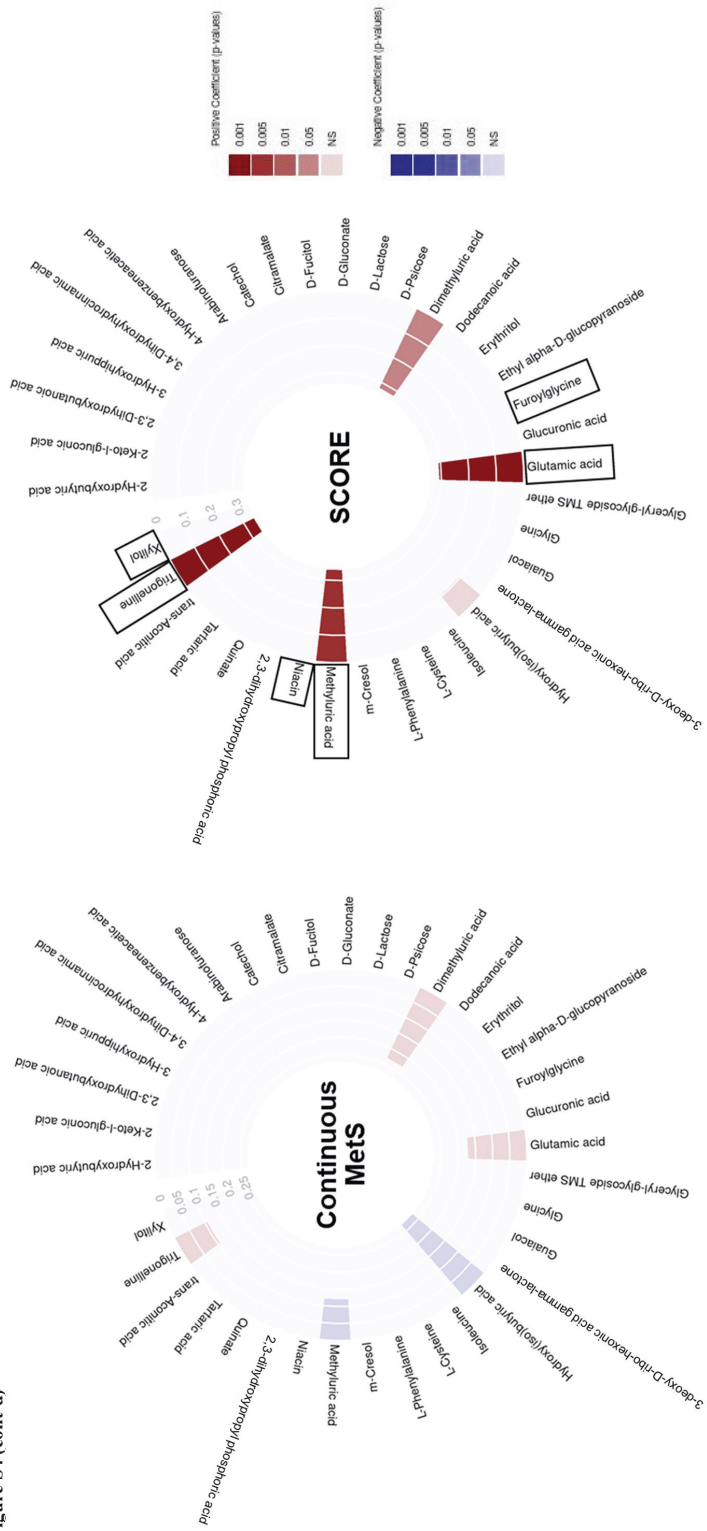

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CHAPTER 7

General discussion

This thesis set out to identify novel candidate FIBs for the habitual intake of fermented foods in a prospective cohort study in the Netherlands (NQplus), as well as to validate several candidate FIBs that were previously identified for dairy foods from non-targeted controlled intervention studies. Further, associations between these FIBs with several cardiometabolic health parameters were examined. Several studies were conducted to meet these objectives with the help of various research tools. These included a systematic review of the literature to identify existing fermented food biomarkers (**Chapter 2**), a comprehensive account of fermented foods consumed in The Netherlands and a statistical comparison of self-report dietary assessment methods for assessing fermented food intake (**Chapter 3**), metabolomics-based approaches to identify and/or validate FIBs of fermented food and dairy intake (**Chapters 4-6**), and association-based approaches to examine the relationships between FIBs with intake and cardiometabolic health (**Chapters 4-6**). In this general discussion, the main findings across each of these chapters will be highlighted and interpreted in the context of the broader literature. Since FIBs of both fermented foods and dairy foods are explored in this thesis, for clarity, the main findings and interpretations for fermented foods will be presented first, followed by dairy foods. A collective reflection on the methodological considerations across all the chapters will then ensue. Finally, the implications of this research together with potential future research directions will be discussed.

Main findings and interpretations – Fermented foods

Types and levels of fermented foods consumed in The Netherlands as assessed by self-report dietary assessment tools

Previously, fermented foods have been estimated to encompass between 5 to 40% of human diets (1, 2), but the origins of these statistics are unclear. Thus in **Chapter 3**, the prevalence of fermented food intake in the Dutch adult diet was evaluated using food lists from the NQplus FFQ and 24-h recalls. To our knowledge, this was the first analysis to quantitate the contribution of fermented foods to human diets, and more specifically, to the Dutch adult diet. Approximately 16 to 18% of foods consumed by this population were determined to be fermented, and a further 9 to 14% of foods consisted of dishes containing a fermented ingredient, or foods that were possibly fermented. The fermented foods consumed primarily consisted of coffee, beer, wine, yeast-leavened bread products, chocolate, cheese, yoghurt, quark, and buttermilk. Several less commonly consumed fermented foods were also captured by 24-h recalls, including certain fermented dairy products (sour cream, crème fraiche, yakult), sausages (salami and chorizo), fermented fish (salted herring, shrimp paste), vegetables (sauerkraut, fermented pickled vegetables), soy (miso, tempeh, soy sauce), and yeast-fermented desserts (doughnuts, pastries). Since these less commonly consumed fermented foods were not reflected in the FFQ food lists, their intakes could not be validated. Nonetheless, the breadth of fermented food products consumed by Dutch adults is an interesting observation in itself. In this context, the analysis in this chapter serves as a starting point for observing future shifts in the types and levels of fermented foods consumed in the Dutch diet, which is relevant for human health research, but also for other research fields (*e.g.*, diaspora of food, global food systems).

In this thesis, information on fermented food intake was taken from FFQs and 24-h recalls. While these self-report dietary assessment tools are often perceived to be inaccurate due to response bias and measurement error, their qualities of being non-intrusive and non-resource intensive still supports their use in large population-based studies. In addition, well-designed FFQs can produce valid and reproducible estimates to rank participants according to their levels of intake. This was observed in **Chapter 3**, where adequate to good ranking ability was achieved for the FFQ compared to multiple 24-h recalls for almost all fermented foods and food groups evaluated (aside from quark and buttermilk). Many of the fermented foods evaluated also demonstrated good agreement in absolute intakes. Nevertheless, the accuracy of self-reported dietary assessment of fermented foods could be improved in future studies. The FFQ relied upon for this analysis was

designed to capture foods commonly consumed in the Dutch diet (≥96% of the absolute level of food intake in the total Dutch diet), but not specifically fermented food products. This was reflected by the relatively low number of unique fermented foods captured in the FFQ food list (39 foods) compared to that of the 24-h recall (247 foods). Thus, there is conceivable value in developing a FFQ specific for fermented foods, which could better capture the habitual intake of fermented foods.

In order for a fermented food-specific FFQ to be effective in accurately capturing the types and levels of fermented foods, researchers must first agree on what foods are considered to be 'fermented'. Just recently in 2021, the International Scientific Association for Probiotics and Prebiotics (ISAPP) convened an expert panel to create a common definition for fermented foods, as "foods made through desired microbial growth and enzymatic conversions of food components" (3). This common definition is a step towards unifying future research on the compositional and health qualities of fermented foods. Additionally, the delineation of fermented and non-fermented foods by the ISAPP expert panel mirrored and validated the classification of fermented foods presented in this thesis. Additionally, consumer misconceptions about fermented foods could be simultaneously addressed. A survey conducted among ~200 university students revealed that nearly two-thirds were unfamiliar with the term "fermented dairy products," and a similar percentage were unsure whether several cultured dairy products were fermented (4). Such unfamiliarity with fermented foods would yield inaccurate estimates of the types and levels of fermented foods consumed in the diet. To circumvent this, improvements in food legislation could make it easier for consumers to identify fermented foods in the marketplace based on clear food labelling (3). Such food labelling should clearly indicate a food or certain food brands as being "fermented", and provide additional nutritional information, such as the presence or absence of live microorganisms, as well as compositional aspects of the fermented food. This information could also be linked to a comprehensive, food composition database to help facilitate future research.

Single-marker vs. multi-marker strategies for estimating fermented food intake

In conjunction with self-report methods, FIBs could act as more objective measures of intake to improve the dietary assessment of fermented foods. In recent years, advancements in the characterization, identification, and validation of FIBs can be partly attributed to the coordinated efforts of the Food Biomarker Alliance (FoodBAll) (5). Among the tasks delivered by the consortium, a series of systematic reviews of FIBs for major food groups were completed (for coffee, tea, sugar-sweetened beverages, cocoa, liquorice, dairy products, egg products, legumes, Allium vegetables, green leafy vegetables, bulb vegetables, stem vegetables, tubers, apples, pears, and stone fruit, nuts, vegetable oils, herbs and spices) (6-15). It is evident from the results of these reviews that: (i) FIBs were not available for many foods, (ii) the majority of identified FIBs were not specific to a particular food, and (iii) the FIBs were not thoroughly validated. To address these limitations, additional intervention studies can be and have been conducted to further identify biomarkers that respond in a time- and dose-dependent manner upon ingestion of a food, and population-based studies to evaluate the robustness of the identified biomarkers in free-living conditions (16). In addition, for non-specific biomarkers, multi-marker panels have emerged as an elevated strategy to capture a broader range of dietary exposure with greater sensitivity and specificity compared to single biomarkers (17, 18). For instance, a urinary multi-marker panel for beer intake consisting of biomarkers from the raw material (hops), malting, and fermentation processes, had excellent performance in distinguishing beer consumption up to 12 hours post-intake (19). The multi-marker approach may also afford greater specificity for other fermented foods, when considering a combination of compounds originating from the raw material and from fermentation processes.

In the systematic review of biomarkers of fermented food intake presented in **Chapter 2**, instead of looking for specific biomarkers, both specific and non-specific compounds were considered in order to support a multi-marker approach in estimating fermented food intake. Compounds identified from the literature search were screened and sorted into FIBs proposed for a specific food based on the food raw material, for a food

group based on overlapping qualities between foods, or for the fermentation process. Out of 301 studies included in the review, the majority reported on FIBs for coffee (69 studies), wine (69 studies), cocoa (62 studies), beer (34 studies), and bread (29 studies). Interestingly, these fermented foods parallel many of those identified from the FFQ in **Chapter 3**, indicating that the most studied fermented foods are also those most commonly consumed in Western/European diets. Alongside food-level markers (*e.g.*, trigonelline for coffee), and food group-level markers (*e.g.*, pentadecanoic acid for dairy intake), several fermentation-dependent markers were identified in the literature review, which were either related to the fermentation process of a particular food (*e.g.*, mannitol for wine, 2-ethylmalate for beer, methionine for sourdough bread and cheese, theabrownins for tea, and gallic acid for tea and wine) or were indicative of more general fermentation processes (*e.g.*, ethanol from alcoholic fermentation, 3-phenyllactic acid from lactic-fermentation). Some of the multi-marker panels outlined in the review have already been demonstrated to be superior to single-markers in predicting food intake. For instance, a combination of ethyl glucuronide (a food group-level marker) and tartaric acid (a food-level marker) performed better than single biomarkers (area under the receiver operating curve (AUROC) of 91 to 92% compared to 67 to 86%) for evaluating exposure to wine in both intervention and observational settings (20). In **Chapter 4**, a multi-marker model consisting of plasma C15, glutamic acid, and isoleucine performed better than single markers for predicting cheese intake (more details provided below). In both these examples, multi-marker approaches indeed appear to be a promising 'successor' to single-marker approaches to effectively capture exposure to as well as levels of fermented food intake. However, many of the proposed multi-marker panels for other fermented foods outlined in the literature review still require further development and validation in future studies. This includes, among other study designs and validation criteria, an exploration of their robustness in free-living populations.

Identification of biomarkers for the habitual intake of fermented foods

The identification of FIBs typically occurs in controlled, intervention studies and subsequently validated in free-living populations with complex, uncontrolled diets. However, direct identification of FIBs in free-living cohorts could yield a list of the strongest FIBs for foods. In **Chapter 6**, non-targeted metabolomics was applied to further identify FIBs of fermented foods in a subcohort of 531 NQplus participants. To ensure comprehensive coverage of the metabolome, plasma and urine samples from these participants were analyzed using both LC-MS and GC-MS. Out of the total of 12 plasma and 26 urinary metabolites identified, the vast majority were found to be discriminant for the intake of fermented beverages (coffee, beer, or wine). One identified metabolite corresponded to the intakes of total fermented cereals/grains, while none of the identified metabolites were discriminant for the intakes of white bread, wholegrain bread, cocoa, or fermented dairy (cheese, yoghurt). This observed imbalance in the metabolites identified for certain fermented foods over others could be attributed to several factors. Firstly, only metabolites significant in two or more statistical tests were selected for identification as a strategy to achieve a number of metabolites that could feasibly be identified, without being biased towards a certain fermented food. Several metabolites discriminant for cheese intake, for instance, were only significant by one statistical method and not prioritized for identification. Secondly, metabolite signals for some foods could be dampened due to shared metabolites with another food. In the case of cocoa, many of the potential food group-level FIBs identified in the literature (caffeine metabolites, hydroxycinnamates, vitamin B3 metabolites) were also found in coffee and could be more strongly associated with coffee intake. Thirdly, the kinetics of the most promising FIBs for a particular fermented food are an important consideration. For dairy foods, including fermented dairy, fatty acids are one of the most widely explored groups of FIBs. However, their levels in blood typically reflects recent dairy fat intake, and may not be detectable under fasting conditions (21, 22). This emphasizes the importance of defining the utility of FIBs based on timeframe of exposure (*i.e.*, recent or habitual intake of a fermented food).

Nonetheless, several of the identified FIBs found to be discriminant for coffee, wine, and beer intake in **Chapter 6** were also captured in the literature search in **Chapter 2**. Notably, these included trigonelline, chlorogenic acid, furoylglycine, and caffeine metabolites (methyluric acid, dimethyluric acid) for coffee intake, and tartaric acid for wine intake. The identification of these previously-identified FIBs in this analysis is simultaneously an indicator of the quality of the data, as well as the strength of the FIB (that it can be identified across different laboratory settings and under different study designs). In addition, a few novel findings included several biomarkers that have not been previously associated with the intake of fermented foods. For instance, urinary 3-deoxy-D-ribo-hexonic acid gamma-lactone was found to be discriminant for coffee intake. This compound is a Maillard reaction product (23), which could have formed during coffee brewing. While some compounds appeared to originate from the food raw material (*e.g.*, niacin and trigonelline for coffee, tartaric acid for wine), others overlapped across different fermented foods (*e.g.*, 4-hydroxybenzeneacetic acid and ethyl α-D-glucopyranoside for both wine and beer). In addition, several metabolites that were identified appeared to be associated with fermentation (erythritol and citramalate) and have been previously detected in multiple fermented food products. Interestingly, the discovery of these metabolites start to parallel the food-level, food group-level, and fermentation-dependent biomarkers described in the systematic review in **Chapter 2**. However, further targeted studies are needed to confirm their fermented food origin (*i.e.*, xylitol from wine and not from non-nutritive sweetener intake), as well as their dose-response relationship in feeding studies with the consumption of different amounts of fermented foods.

Common metabolites from food fermentation and gut microbiota metabolism

Some fermentation-dependent FIBs are noticeably absent from the list of most discriminant metabolites identified from non-targeted analysis in **Chapter 6**. This could be due to many factors, among which and worth discussing is the overlap between metabolites derived from food fermentation and those from the gut microbiota. Undigested carbohydrate and protein from multiple food sources can be transformed by the gut microbiota to produce a diverse range of metabolites, including short-chain and branched-chain fatty acids, ammonia, (biogenic) amines, and phenolic compounds (24). Other metabolic activities of the gut microbiota parallel those of microorganisms involved in food fermentation, including the production of bioactive food components (*e.g.*, isoflavanoids, flavanoids and plant lignans) and vitamins (*e.g.*, vitamin K2) (25, 26). Evidently, metabolites produced by both food fermentation microorganisms and the gut bacteria can influence host physiological processes and influence the risk of disease. However, this interplay also makes it difficult to distinguish metabolites that are FIBs for fermented food intake. For instance, the lactic acid bacteria metabolite 3-phenyllactic acid was previously identified in serum and urine of volunteers consuming a single dose of cheese (27, 28). In **Chapter 4**, a targeted evaluation of 3-phenyllactic acid in plasma and urine yielded non-significant associations between 3-phenyllactic acid with cheese intake. A biological explanation for this lack of association could be the co-origin of (D)-3-phenyllactic acid from fermented foods and gut microbiota metabolism of food sources of phenylalanine (29). Additionally, the L-isomeric form of 3-phenyllactic acid can also be produced endogenously from phenylalanine, which is indicative of inborn errors of metabolism (phenylketonuria) (30). Further studies to explore the quantitative contribution of (D)-3-phenyllactic acid and similar metabolites originating from fermented foods or gut microbial metabolism using labelled isotopic standards could help clarify the status of these metabolites as FIBs for fermented foods.

Exploring strategies to group fermented foods

Another approach to better identify fermentation-dependent metabolites is exploring strategies to group fermented foods. Some metabolites may not be strong enough to distinguish the relatively low intakes of individual fermented foods, but may be more potent for detecting larger intake levels afforded by a fermented food group. Additionally, there could be added value in exploring how a dietary pattern of fermented food consumption (rather than individual foods) impacts health. In **Chapter 3**, dry matter was explored as a

method for unifying fermented foods with different matrices (*e.g.*, liquid, solid) using information on water content that was readily available in the food composition tables. Using this method, metabolites were identified based on g/day and g dry matter/day intake levels for total fermented beverages, total fermented cereals/grains, and total fermented dairy groups. While these groups were generated to be consistent with the groups used in the literature search (**Chapter 2**) and FFQ validation (**Chapter 3**), it is recognized that some groups may be more effective than others for the identification of FIBs of fermented foods. For instance, while the total fermented beverages group consisted of a range of unique foods with few shared qualities (aside from the presence of alcohol in wine and beer), other groups such as fermented dairy were more homogenous (*e.g.*, products derived from milk via lactic acid fermentation). To this end, another grouping strategy was considered that involved biomass (*e.g.*, CFU/day) based on the levels of microorganisms used in fermentation of foods or contained in ready-to-eat fermented foods. Information on the types of microorganisms involved could also allow fermented foods to be grouped based on common fermentation processes (*e.g.*, lactic acid fermentation) or based on the presence/absence of live microorganisms. Unfortunately, information on the type and levels of microorganisms could not be obtained or calculated from self-report dietary intakes data used in this thesis. Documentation of such information would be helpful to the discovery of fermentation-dependent biomarkers as well as in future studies exploring the health impacts of fermented food consumption.

Associations between potential FIBs of fermented foods with cardiometabolic health

Traditionally, fermentation has been largely regarded as a technique to preserve foods; thus, the consumption of fermented foods for health benefit is still a developing research area. Evidence from human studies have suggested that consumption of fermented foods may have a favourable impact on cardiometabolic health. For instance, a recent meta-analysis of cohort studies showed that fermented dairy intake was inversely associated with cardiovascular risk (myocardial infarction, coronary heart disease, stroke, other cardiovascular events) (odds ratio (OR) 0.83, 95% CI 0.76–0.91) (31). In addition, there is also a biological rationale for exploring the health impacts of fermented food intake. Dietary compounds originating from or enriched by the fermentation process may impact diverse metabolic pathways that collectively govern cardiometabolic disease prevention and management. For example, cheese contains αs1- and β-casein peptide fragments (produced from milk casein during fermentation of milk), which have ACE inhibitory activities and could help to maintain blood pressure levels (29). Thus, beyond identifying metabolites as FIBs for fermented foods, there is also an opportunity to explore if these metabolites have a modulatory effect on health outcomes. In **Chapter 6**, associations between identified candidate FIBs of fermented foods with cardiometabolic risk parameters were examined. In the fully-adjusted regression model, 30 metabolites were found to be significantly associated with various CMD risk parameters (20 positively and 10 negatively associated). After adjusting for multiple comparisons, 10 associations remained significant, including between plasma glutamic acid with BMI and waist circumference, as well as a handful of plasma and urinary metabolites (xylitol, glutamic acid, trigonelline, niacin, furoylglycine, and methyluric acid) with SCORE. The magnitude of the associations appeared to be weak. Nonetheless, there can be several interpretations of these findings. Firstly, as this analysis was conducted in a cross-sectional setting, these results are more hypothesis-generating than implying causation. A number of factors were considered in the adjustment of the models that could confound the association (*e.g.*, sex, age, smoking status, education, dietary factors, as well as with and without adjusting for BMI), but this does not exclude the possibility of other relevant confounders. Secondly, since the associations were relatively neutral, the perceived cardiometabolic health benefits of fermented foods could be attributed to other components of fermented foods beyond these identified metabolites (*e.g.*, bioactive peptides, or to live microorganisms). Overall, the potential of exploring FIBs of fermented food intake as markers to inform the risk of CMD is an interesting avenue for future research, but more work is required before tangible future recommendations can be made regarding the benefits of fermented food products on cardiometabolic health.

Main findings and interpretations – Dairy foods

Alongside general fermented foods, dairy foods (encompassing both fermented and non-fermented dairy) were evaluated in this thesis. Dairy products constitute an important part of European diets, and encompass a diverse range of foods derived from milk that are heterogeneous in nutritional composition. It is generally agreed that dairy provides an important source of nutrients including calcium, selenium, iron, zinc, copper, folic acid, and vitamins (C, D, B2, B12), which help to promote bone health throughout the lifespan (32, 33). However, the health impacts of consuming dairy foods as a source of dietary fatty acids is far more contested, due to possible adverse effects of saturated and *trans* fatty acids on cardiometabolic disease risk (33, 34). Recent shifts in the paradigm from viewing dairy products as a homogenous group for delivering specific nutrients to examining the whole dairy matrix indicates that distinct dairy foods (*i.e.*, milk, cheese, yoghurt) may have varying effects on cardiometabolic health. Fully validated FIBs (single or multi-marker) have yet to be determined for distinct dairy foods, and their elucidation may help provide updated dietary guidelines for dairy foods that deviate from a 'one size fits all' approach.

Types and levels of dairy foods consumed in The Netherlands as assessed by self-report dietary assessment tools

In **Chapter 3**, the types and levels of dairy foods consumed in The Netherlands was also documented. The predominant dairy foods included (as assessed by an FFQ): cheese, yoghurt, buttermilk, quark (fermented dairy products), as well as milk, butter, cream, and ice cream (non-fermented dairy products). The mean total dairy intake of the NQplus population was 323 g/day (13.7% of total daily energy intake). This intake level is in line with the nutritional recommendations set by the Health Council of The Netherlands to consume sufficient levels of dairy products per day (*e.g.*, 2-3 portions/day, ~300 g/day) (35, 36). Similar to the evaluations of fermented foods, the FFQ provided valid and reproducible intake estimates for multiple regularly consumed dairy foods and groups. Adequate to good agreement in both ranking ability and absolute intakes was determined for total fermented dairy, cheese, and butter intake assessed by the FFQ compared to multiple 24-h recalls. For total non-fermented dairy, yoghurt, and milk, good ranking ability of participants into their levels of consumption could be established, albeit poor agreement in absolute intakes. For the less commonly consumed fermented dairy foods quark and buttermilk, and non-fermented dairy foods cream and ice cream, acceptable relative validity between the two methods could not be established, and self-reported estimates should be taken with caution.

Interestingly, this analysis also revealed that intakes of total fermented dairy products was slightly higher than non-fermented dairy products (171 vs 153 g/day). Fermented dairy products also represented the largest group of fermented foods with live microorganisms consumed by this population, which may have important implications for human health. However, the compositional and microbiological qualities of different fermented dairy products is difficult to capture in self-report dietary assessment. As detailed in the general introduction, there is incredible diversity in the types of microorganisms used in the fermentation of dairy foods (at both the genus and strain level). Additionally, levels of microorganisms in final cheese and yoghurt products have been shown to vary from non-detectable levels to $>10^9$ CFU/g or mL, depending on the manufacturing, processing, and storage conditions (37). Beyond microbiological diversity, the composition of fermented dairy foods can also be affected by a myriad of factors, such as the animal source of milk, seasonal variation in animal grazing conditions, pasteurization, and length of fermentation/maturation (38, 39). In the case of cheese, variability in microorganisms and metabolites have also been observed in different parts of the cheese matrix (rind, core) (39). While these meta-data could be important to document for differentiating the health impacts of fermented dairy products with different qualities, is it difficult to do so effectively in self-report dietary assessment. The FFQ used in this thesis only provided information on fat content for yoghurts

and cheese products – a design feature which facilitates easy completion and avoids (to some extent) wrongful misclassification by participants. Alternatively, the development of databases containing a comprehensive and integrated analysis of the composition of fermented dairy foods, as well as their detection in biosamples after consumption (biomarkers) could help verify self-reported food intake.

Biomarkers of dairy (fat) intake

Several fatty acids have been proposed as biomarkers of dairy fat intake, some of which also associate with general dairy intake. Among the most well studied FIBs for dairy (fat) intake are the odd-chain fatty acids C15 and C17. These fatty acids are synthesized by intestinal bacteria in ruminants, and have been used as 'validated' biomarkers of dairy fat intake in association studies linking dairy intake to cardiovascular disease, stroke, and type II diabetes (40-43). However, FIB validation is an iterative process and new research findings could alter the validation status. For instance, Ratnayake (44) and others have pointed out that the concentrations of C15 and C17 in dairy fat are very low (C15 at 1.0%, C17 at 0.6%) and can co-elute with other fatty acids if the analytical conditions are not optimized. Additionally, they are widely detected in many foods other than dairy (fish, animal fat, and even some vegetables and seaweeds) (44). Thus, while C15/C17 have been demonstrated to be associated with dairy (fat) intake, studies examining associations between plasma concentrations of these fatty acids and disease outcomes should be considered with caution, as these associations may depend on the other foods typically consumed in the diet of the population(s) in which the biomarker was identified/validated.

In **Chapter 4**, the associations of C15 and C17 in fasting plasma with dairy intake were also evaluated. The results showed that C15 was significantly positively correlated with total dairy, total fermented dairy, and low-fat fermented dairy intake (r_s 0.16 to 0.24), but curiously not high-fat dairy groups. The lack of associations with high-fat dairy groups may also be explained by the fact that this group was based on individual dairy foods (milk, cheese, yoghurt) with high fat content, rather than based on absolute quantities of dairy fat. Additionally, consumers eating low amounts of high-fat dairy could simultaneously be eating high amounts of low-fat dairy, which further blurs the association between C15 and dairy groups. Associations between C17 and fermented dairy groups were also observed, but were weaker than C15 – a finding which is aligned to previous studies in the literature, and could be due to the associations found between C17 with dietary fiber intake (45, 46). In the quantitative targeted analysis of milk-derived fatty acids described in **Chapter 5**, stronger correlations were observed between C15 and a larger number of dairy groups: total dairy, high-fat dairy, total fermented dairy, low-fat fermented dairy, high-fat non-fermented dairy, cheese, and yoghurt (r_s 0.18 to 0.25). In addition, the strongest correlation was observed for dairy fat intake (r_s 0.26), confirming previous reports of the utility of C15 as a biomarker of dairy fat. Conversely, no significant associations were reported for C17. These modest positive associations between C15 (and to a lesser extent, C17) suggest that, while these fatty acids are now known to be non-specific and not appropriate as sole indicators of dairy (fat) intake, they could remain useful as a 'benchmark' to help contextualize the associations and validation performances of other proposed FIBs for dairy intake.

Several other fatty acids have also been reported to be associated with dairy fat intake, including C14:0, C14:1, C17:1, *trans*-C16:1n-7, *trans*-C18:1(n-7), and CLA (8, 47, 48). These fatty acids were also among those quantified in **Chapter 5**. In the fully-adjusted regression model, *trans*-C16:1n-7 (C16:1 t9) was positively associated with intakes of high-fat dairy, dairy fat, high-fat fermented dairy, and cheese, and sum of CLA was positively associated with dairy fat intake. However, the other fatty acids were either not detected in at least a third of participants (C14:0), not significantly associated (C14:1, *trans* 18:1n-7), or were inversely associated with dairy intake (C17:1). In addition, these fatty acids were not found to be discriminant for the intake of milk, cheese, or yoghurt in previous FIB identification studies conducted for these dairy foods (27, 28, 49, 50).

The findings from these chapters lend support to previous research on fatty acids as FIBs of dairy (fat) intake, in particular C15, *trans*-C16:1n-7 and CLA. However, the modest associations observed indicate that there is still room to improve the accuracy and precision of the biomarkers, possibly through exploiting multi-marker panels. To this end, non-targeted metabolomics and lipidomics studies conducted on dairy foods and on biosamples after dairy consumption can reveal a wider panel of metabolites and lipids which can help in the selection of candidates for exploration in a multi-marker model (51, 52).

Single-marker vs. multi-marker strategies for estimating dairy food intake

Aside from dairy fat intake, FIBs for the intake of specific dairy foods have not yet been fully exploited. The Netherlands has one of the highest dairy consumption per capita in the world (53, 54), which makes the Dutch population ideal for identifying and validating candidate FIBs of dairy food intake. In **Chapter 4**, the performance of single-marker and multi-marker strategies in estimating the intake of dairy foods was evaluated in the NQplus population. The candidate FIBs were previously-identified in the plasma and urine metabolomes of healthy adults following milk, cheese, and yoghurt consumption in postprandial and/or short-term intervention studies, and mainly consisted of lactose metabolites, oligosaccharides, fatty acids, amino acids, and indoles (27, 28, 49, 50). For milk, the best multi-marker model generated from stepwise regression that also accounted for common covariates (urinary galactose, galactitol, sex, body mass index, and age; correlation between actual and predicted intakes (r_{ap}) of 0.20, mean absolute error (MAE) of 92 g/day) was found to be more effective in predicting milk intake than the best single-marker model (urinary galactitol; r_{ap} 0.17, MAE 94 g/day). Similar results were obtained for the best multi-marker model for cheese intake (plasma pentadecanoic acid, isoleucine, and glutamic acid; r_{ap} 0.16, MAE 17 g/day vs. no significant single-marker model). Meanwhile, no significant associations were observed for yogurt, which could be attributed to the non-specific nature of the biomarkers identified for this dairy food (primarily amino acids).

This is the first report to explore multi-marker models for capturing the intake of specific dairy foods. Broadly speaking, the main findings from **Chapter 4** can be summarized into two conclusions. The first is that the development of multi-marker panels is an appropriate strategy to capture the intakes of dairy foods with an improvement over single-markers. This has also been demonstrated previously for dairy fat intake, where summed combinations of fatty acids (C15 and *trans*-C18:1(n-7) in fasting plasma triglycerides (R^2 = 0.128), and C14:0, C15, trans-C18:1(n-7) and CLA (R^2 = 0.143) in phospholipids) resulted in stronger predictions of dairy fat intake over single fatty acids (55). The second is that the best individual FIBs, and the best combinations of FIBs, have yet to be elucidated. This includes an elucidation of separate panels for the short-term and habitual intake of dairy foods. The tryptophan metabolites indole-3-lactic acid and indole-3-acetaldehyde illustrate this point. In a previous non-targeted metabolomics study of yoghurt intake, these indoles showed significant post-prandial responses to yoghurt intake, but were almost non-detectable in serum 6 hours after consumption (49). Additionally, they were not significant in fasting serum after daily yoghurt intake for two weeks. Thus, they may not be suitable as habitual markers, but may be valid as a single or combined marker to reflect acute yoghurt intake.

To complement the targeted metabolomics analysis, a semi-exploratory study was performed in **Chapter 5** to examine the associations between 67 milk-derived free fatty acids with dairy fat intakes, as well as different dairy groups and dairy foods. In the full-adjusted model, the strongest associations were observed for dairy fat intake included C16:1 t9 (standardized β (Std. β) = 4.9, SE = 1.6, R^2 = 0.2, raw $p \leq 0.05$), which was also associated with high-fat dairy, high-fat fermented dairy, and cheese intake. Significant associations between C15 with dairy fat and high-fat dairy intake were also observed (albeit with lower effect size compared to C16:1 t9), while no significant associations were observed for C17. Several long-chain saturated and unsaturated free fatty acids were also found to be positively associated with several dairy groups and dairy foods which have not been widely reported (C15 *iso*, C17 *iso*, C18:1 t13+c6+c7+u, C18:1 u1, C18:2 c9t11+u,

C18:2 t9t12, C18:2 u3, C20:1 c11, C20:3 c8c11c14, C20:4 c5c8c11c14, C20:4 c8c11c14c17, and C22). While the majority of these free fatty acids had low concentrations in plasma similar to C15 and C17 (≤0.7 mg/mL), the GC-FID targeted quantitative method is well developed to be able to efficiently separate different fatty acids and their isomers (56, 57). These individual fatty acids should be further explored in multi-marker panels as alternative or complementary FIBs for different dairy groups or foods.

As mentioned above, several multi-marker models consisting of summed fatty acids have been previously explored for dairy fat intake. Pranger *et al.* (55) observed that summed plasma triglyceride concentrations of C15 and *trans*-C18:1(n-7), and plasma phospholipid levels of C14:0, C15, *trans*-C18:1(n-7) and CLA, best captured dairy fat intake over each of the single fatty acids. In another study, the authors considered the summed plasma phospholipid levels of C15, C17, and *trans*-palmitoleic acid (C16:1 t9) to be a biomarker of dairy fat intake, based on the evidence each individual biomarker and the high degree of inter-correlation between these biomarkers (58). In **Chapter 5**, associations were conducted between 21 summed fatty acid groups with intakes of dairy fat, dairy groups, and individual dairy foods. This also included an evaluation of summed C15 and C7, and summed C15, C17, and C16:1 t9, which were not found to be significantly associated with dairy fat, dairy groups, or individual dairy foods in the fully-adjusted model. However, sum of CLA was found to be positively associated with dairy fat intake, while sum of *trans*-C18:2 with CLA was positively associated with cheese intake. CLA (primarily the *cis*-9, *trans*-11 form in milk) is largely synthesized by bacteria in ruminants from unsaturated fatty acids; however, it is not regarded to be specific to cheese intake but rather a marker for the general intake of ruminant foods (8). Moreover, the use of summed fatty acids as multi-marker models is not without its limitations. Summation is a linear combination that assumes that all fatty acids in the sum predict dairy fat intake equally well, which is almost never the case. Since the goal of this chapter was not to compare the associations of single or combined fatty acids for capturing dairy intakes, different combinations of the 67 milk-derived free fatty acids were not fully explored. However, there is a rationale for using the strongest individual fatty acids from this analysis in a future multi-marker panel. These multi-marker panels should include complementary biomarkers from different metabolite classes that capture different aspects of a food. For dairy foods, this could include fatty acids, non-fatty acids, fermentation-derived metabolites (if appropriate), metabolites from processing, additives, as well as known physiological and genetic targets that could influence the utility of the biomarkers for specific sub-populations.

Associations between dairy biomarkers and cardiometabolic health

Several studies have been conducted associating dairy fat biomarkers (C15, C17, and C16:1 t9) with CMD risk factors and disease incidence, such as with incident type II diabetes (43) and incident stroke (42). However, relatively little is known about the wider panel of milk-derived fatty acids and how they individually and collectively associate with different CMD risk parameters. In **Chapter 5**, in the fully-adjusted regression model, 33 free fatty acids were found to be associated with CMD risk parameters. Several long-chain saturated (C16-C19) and unsaturated FFAs (*e.g.*, DHA) as well as several summed fatty acids (*e.g.*, omega-3 and omega-6 fatty acids) positively associated with plasma total cholesterol, LDL-cholesterol, serum triglycerides, and SCORE; the strongest associations were observed for C18:1 c15 with plasma total cholesterol and LDL-cholesterol. On the other end, several long-chain saturated and unsaturated FFAs were also found to be negatively associated with waist circumference, serum triglycerides, and plasma LDL-cholesterol. These findings suggest that the metabolic impacts of dairy consumption on plasma free fatty acids is bidirectional, and could have implications for further understanding how dairy components contribute to overall CMD risk. Interestingly, in contrast to candidate FIBs for fermented beverages (coffee, beer, and wine) which were mainly associated with anthropometric parameters, here, associations were mostly observed between dairy fatty acids and blood lipid parameters. While clear associations between self-reported dairy intake and CMD risk factors

were not observed in this study, a larger population (or cohort with a greater gradient of CMD risk) is warranted to confirm these neutral findings, or to address the limitations of the current study design.

Methodological considerations

Population-based study design for the identification and validation of FIBs

Several study designs have been used for the identification of FIBs, the most common of which is a well-controlled, acute dietary intervention study where participants consume a single test food/meal (59). This study design ensures a cleaner selection of the most discriminant metabolites for a specific food, and analysis of post-prandial samples collected at set intervals following consumption can provide valuable information on the dose-response of the FIBs (27, 28, 49, 50, 59). In the current thesis, identification of FIBs for fermented foods was achieved using biosamples from an existing free-living cohort (NQplus), which has distinct advantages and limitations over an intervention approach. For instance, although metabolite changes are more difficult to detect in cohort samples, those detected can be considered more powerful and reliable indicators of fermented food intake since they are identified in the context of a highly variable diet (60). This was exemplified by the identification of known coffee intake biomarkers (*e.g.*, quinate, trigonelline), from the non-targeted analysis conducted in **Chapter 6**. The use of existing cohort samples to identify these FIBs can also be a more efficient approach than conducting an intervention study for each food of interest. For example, in one study, proline betaine and flavanone glucuronides (several known biomarkers of citrus fruit intake) were equally identified in the urine metabolomes of participants who had consumed an acute dose of orange or grapefruit juice, regularly consumed orange juice for one month, and between regular low- and high-consumers of citrus (60). Further, although intervention studies may be more suited for identifying new biomarkers, it is critical for the validation of these biomarkers to be conducted in a free-living cohort with complex, uncontrolled diets (16). For example, in **Chapter 4**, the robustness of previously-identified FIBs for milk, cheese, and yoghurt intake were evaluated in a free-living cohort. While these FIBs were identified postprandially following an acute ingestion of the dairy foods and could thus reflect recent intake, many of them were not robust enough to discriminate the habitual intake of these dairy foods.

Qualities of biosamples collected from population-based studies

A related consideration for the use of population-based studies to identify and validate FIBs relates to the differences in the collection and storage times of biosamples. Large-scale population-based studies are typically designed for multiple data extraction and analyses (due to the amount of effort and cost involved to set up such a study). Thus, in contrast to intervention studies where post-prandial blood and/or urine are collected and analyzed fairly quickly, cohort biosamples are typically collected under fasting conditions, at one timepoint, and then stored in the biobank for many years. The use of these biosamples presents challenges for biomarker identification. Certain metabolites may degrade during the harsh sampling, handling and long-term storage conditions. Therefore, these metabolites are not detected during FIB identification or validation, even if they are promising candidates. In addition, candidate FIBs previously detected under postprandial conditions and have a short half-life may not be detected in fasting biosamples (*e.g.*, FIBs for yoghurt targeted in **Chapter 4**). The stability of FIBs in biosamples under long-term storage conditions, as well as their analytical performance and reproducibility across laboratories with different quantification methods, require dedicated studies (61). Interestingly, since 24-h urine samples were collected for NQplus (instead of fasting urine), in **Chapter 6**, a larger number of metabolites were significant for and identified in urine compared to plasma. This indicates that the use of 24-h urine or biosample pools in population-based studies could be a more effective strategy to the use of fasting samples in detecting a greater range of metabolites.

Metabolomics approaches for the identification and validation of biomarkers

Metabolomics is a versatile tool that permits the comprehensive profiling of thousands of metabolites in biofluids with great breadth and precision. These metabolites contain valuable information reflecting new gene functions, altered metabolic pathways, food-host-gut microbiota interactions, and food intake (61). The choice of the analytical approach varies on the goal of the study. Different metabolomics approaches have been described in this thesis, namely a non-targeted semi-quantitative approach (LC-MS, GC-MS) for the identification of fermented food FIBs (**Chapter 6**), and a targeted semi-quantitative (LC-MS, GC-MS) approach for evaluating the robustness of previously-identified FIBs (**Chapters 4, 6**). Further, a targeted quantitative (GC-FID) approach was used for fatty acid analysis (**Chapters 5**). For biomarker identification, a combination of metabolomics platforms permits a comprehensive coverage of the metabolome based on the strengths of each method. However, the dose-response relationship between fermented food (or dairy) intake and the biomarker (single or combined) will be fully elucidated only by conducting human intervention studies with several doses of these foods and quantitative methods of analysis, allowing the establishment of calibration curves and integration of biomarkers from different platforms.

Several challenges faced in this thesis were related to metabolite identification, which is regarded as the bottleneck in metabolomics. For GC-MS, the stability of the machinery permits the use of reference libraries to offer accurate identifications for many metabolites, even in the absence of analytical standards. However, for LC-MS, identification is more difficult. In **Chapters 4 and 6**, all LC-MS metabolites were analyzed using positive mode, to align with previous research studies, and to make use of the optimized settings by which the widest range of metabolites could be detected. However, running the same samples through negative mode could have been beneficial to detect different metabolites (extending the number of metabolites measured) but also verify the same molecules (providing more information on their identity). Nevertheless, identification of metabolites for the LC-MS was aided by performing MS/MS fragmentations using different settings for plasma and urine QC pools, as well as in several 'high consumer' samples. This resulted in a larger number of fragmentation spectra (20-30% increase) that could then be compared to databases in order to help confirm the suggested identities of the metabolites.

A further challenge in metabolomics relates to the integration of datasets across multiple metabolomics analytical platforms. In **Chapter 4**, candidate FIBs for milk, cheese, and yogurt were targeted using the same metabolomics platforms from which they were originally identified (LC-MS and GC-MS, or GC-MS as a proxy for metabolites identified previously using NMR). Thus, the multi-marker models generated were also biosample- and platform-specific. Standardized protocols are lacking for how to effectively combine metabolites detected across different biosamples and analytical platforms, which is an area in need of development. Beyond metabolomics-metabolomics data integration, further guidance on how to integrate metabolomics data with genomics (metagenomics-microbiome), transcriptomics, and proteomics data would offer unparalleled opportunities to enhance current understanding of biological functions, elucidate their underlying mechanisms, and uncover hidden associations between different omics variables (62).

Selection of statistical and prediction models

A common thread throughout the chapters in this thesis is the comprehensive comparison of different statistical methods. In **Chapter 3**, four different statistical methods were outlined to evaluate the relative validity of the FFQ against multiple 24-h recalls: Spearman's correlation, Bland-Altman, quintile cross-classification, and percent difference in absolute intakes. The use of these multiple methods reveals different facets of validity that would not be achieved with a single method (63). For example, the results of quintile cross-classification showed that the FFQ was able to classify some fermented and dairy foods correctly into levels of intakes, but percent difference revealed poor agreement for absolute intakes. Similarly, in **Chapter 6**, a combination of univariate (Spearman's correlations, Kruskal-Wallis) and multivariate (PLS-DA, Random

Forest) techniques were applied to select the most discriminant metabolites as potential FIBs for fermented foods. Data analysis in metabolomics is generally dominated by multivariate methods due to the large number of variables measured; however, univariate methods are gaining momentum partly due to their ease of application and interpretation (64). In fact, the predominant method for the analysis of metabolomics data in epidemiology-based studies of biomarker discovery and disease etiology is univariate regression (65). The use of both statistical approaches can result in the identification of complementary FIBs (64). Indeed, a large number of significant metabolites were revealed following the statistical analysis in **Chapter 6** (586 plasma and 151 urinary metabolites from GC-MS, 110 plasma and 4473 urinary metabolites from LC-MS).

In **Chapters 4 and 5**, various regression models (generalized linear regression, step-wise regression, multiple linear regression, and restricted cubic spline regression) were explored as predictive models for estimating dairy intake using single or combined biomarkers (or individual and summed fatty acids). However, in each case, associations were examined over agreement. Typically, a binary classification method, such as area under the receiver operator curve (AUROC), is used to observe whether the FIB can correctly classify consumers and non-consumers of a food. This has been applied for single- and multi-marker panels developed for banana (66), beer (19), wine (20), cocoa (67), and fruit and fruit juice intake (18, 68). In one study, a summed combination of biomarkers (proline betaine, hippurate, and xylose) showed an improved ability to classify individuals into 3 categories of self-reported fruit intake compared to each biomarker alone (18). However, the ability of multi-marker panels to accurately estimate a continuous range of food intakes is not well explored. To this end, further development of appropriate statistical methods to examine the agreement between self-reported food intake and levels of FIBs on a continuous scale (for example, as proposed by Obuchowski (69) for diagnostic tests) would be highly valuable for the further validation of FIBs.

Measurement error in self-report and biomarker-based dietary assessment

It is well recognized that self-report dietary assessment methods suffer from measurement error. These can be random errors, which reduces the precision of intake estimates and loss of statistical power to detect potential associations, or systematic errors, which reduce the accuracy of intake estimates and over or underestimate diet-health associations (70-72). Random errors, such as those related to day-to-day variation in food intake, can be reduced by taking repeated measurements (*e.g.*, multiple 24-h recalls per person). For example, in **Chapter 3**, all of the available 24-h recalls completed by a given participant (2-8 recalls, most had completed ≥3 recalls) were averaged in order to smoothen day-to-day variation and achieve habitual intake estimates akin to the FFQ. On the other hand, systematic errors caused by inaccuracies in participant responses (*e.g.*, overreporting the consumption of healthy foods and underreporting of junk foods), can introduce bias and are unable to be addressed adequately with an increased number of measurements (71).

In this thesis, FIBs are presented as a complementary dietary assessment strategy that is less prone to systematic measurement error than self-report methods, to achieve intakes estimates closer to 'true' intake values. Additionally, multi-markers that are more specific for the intakes of a certain food may perform better than single biomarkers in achieving this goal. However, due to the nature of the NQplus data, the dates of FFQ completion and biosample collection did not coincide. The identification and validation of FIBs was therefore performed on biosamples collected within 14 days of completing an FFQ, to achieve a large yet reasonable number of participants with biosamples collected within the reference timeframe of the FFQ. This timeframe is still relatively large and can present challenges to the identification of FIBs that are sensitive to daily fluctuations in dietary intake. Thus, in **Chapter 6**, the stability of the identified FIBs (for coffee, wine and beer) was explored by comparing the correlations between FIBs and self-report intakes with increasing time between biosample collection and FFQ completion. The magnitude and significance of the correlations were maintained despite increasing timeframes (within 30, 90, 180 days, and greater). This analysis suggests that these FIBs could be relatively stable for assessing the habitual intake of these foods.

Beyond biomarkers, other technology-based methods have been proposed to reduce measurement error and improve the accuracy of dietary intake assessments (for both research and consumer use). These range from web-based translations of traditional paper-based methods, to meal photography linked with a smartphone app (73). These methods can be cost-effective, fairly easy to complete, and provide rapid feedback (73). Eventually, technology-based methods may even surpass FIBs as a more effective strategy to gain accurate information on portion size estimation. However, the caveat is that such methods are not able to satisfy the biological knowledge gained along the search for FIBs.

Moreover, regardless of the method (self-report, biomarker, technology-based), there is no way to assess dietary intake with perfect precision, as all methods suffer from measurement error. As stated by Beaton *et al.* (74): "...there is not, and probably never will be, a method that can estimate dietary intake without error...different types of error have different effects in analysis and interpretation." Thus, in addition to developing and validating new methods, the error structure (nature and magnitude of error) of dietary data must be assessed (for instance, correlation attenuation factors described in **Chapter 3**). Of course, the tolerance for the amount of measurement error also depends on the research question: whether it is important to rank individuals into levels of intake, or to achieve congruency in absolute intakes.

Implications and future research directions

This thesis adds to the current state of the literature on FIB identification and validation, in particular for fermented foods and dairy foods. At the same time, several knowledge gaps were revealed, which limits the current application of FIBs in improving the dietary assessment of fermented foods and dairy foods, and understanding their role in (cardiometabolic) health. In the ensuing sections, several future research directions will be presented, along with their implications for public health, the food industry, and consumers.

Expanding the discovery of FIBs for different types of fermented foods

A common knowledge gap among the chapters in this thesis stems from a bias in the literature for investigating a narrow list of fermented foods. In the systematic review of FIBs for fermented foods (**Chapter 2**), the vast majority of studies were conducted for coffee, wine, cocoa, beer, and bread, while only a smattering of other types of fermented foods were represented. For some foods, such as fermented soy, there appears to be ample research but the studies primarily target a list of food group-level biomarkers (*e.g.*, isoflavones found in all soy products), whereas FIBs specifically associated with fermented soy intake were not investigated. This bias was further reflected in the fermented foods assessed by the FFQ (coffee, beer, wine, yeast-leavened breads, chocolate, cheese, and yoghurt) (**Chapter 3**), and consequently in the identification of FIBs using these self-reported fermented food intake groups (**Chapter 6**). While these are common foods consumed in Western/European diets, due to globalization, many of the nutritious and delightful fermented food products that are indigenous to other parts of the world are now available locally. Thus, the current literature on FIBs of fermented foods presents an 'incomplete' picture of the diversity of fermented foods consumed in modern globalized diets. To address this, further discovery-driven studies on the identification of FIBs for less common fermented foods and condiments in Europe (*e.g.,* sourdough, sauerkraut, salami, Worcestershire sauce) and globally (*e.g.,* kombucha, kefir, tempeh, kimchi, soy sauce) are warranted.

Better understanding the composition of different fermented foods and their documentation in food databases

Fermented food products are complex and multi-faceted. The composition of fermented foods reflects untransformed compounds from the raw food substrate, transformed raw material compounds by the fermentation process, and novel metabolites produced by fermentative microorganisms. Additionally, the presence of live microorganisms (or even inactivated microorganisms) presents a unique additional

compositional layer (3, 75). Metabolomics and metagenomics analyses coupled with bioinformatics and machine learning could better capture the molecular composition of fermented foods. Concurrently, documenting the composition of different fermented foods and their meta-data in food composition databases (new or existing) is a critical step. Food composition databases are widely used in nutrition and health research to provide information on the nutritional content of foods. However, current food composition databases only report a limited number of nutrients out of the thousands of distinct chemicals in our food (76). Documenting the composition of fermented foods could open new research avenues for understanding how their consumption affects health and disease. Additionally, such information could also benefit food industries to formulate products with improved nutritional qualities.

Further development and validation of multi-marker panels for capturing fermented food (and dairy food) intake and association studies in larger cohorts

Both of the abovementioned future research directions (expanding the repertoire of FIBs for fermented foods consumed globally and further understanding their composition) directly benefits the development of future multi-marker panels. For instance, one lesson derived from this thesis is that while a combination of biomarkers can have the potential to capture intakes of specific foods with greater efficacy over single markers, the best combinations of FIBs have yet to be fully elucidated. This was observed for many FIBs identified for fermented foods as well as dairy foods. Additionally, a further study could be conducted to validate FIBs identified for fermented foods and dairy foods in a larger population where biosample collection occurs as close as possible to completion of self-report dietary assessment (*e.g.*, within one day). Given that the NQplus population is relatively healthy, investigation in a larger cohort (or nested case-control study) would also present a larger gradient of cardiometabolic risk between participants, which may be useful to better observe associations between fermented food intake, FIBs, and cardiometabolic health. Further, quantification of the most promising FIBs could help in order to use these biomarkers to calibrate self-reported fermented food intakes (77). Expanding such research could eventually have an impact on public health, such as through refining dietary guidelines for fermented foods as well as for specific dairy foods.

Better understanding human variability for improving personalized nutrition

A further research area could be to examine how human variability affects the effectiveness of FIBs for certain foods. For instance, in **Chapter 4**, the prevalence of the lactase persistent genotype (ability to digest lactose in adulthood) was examined, and its influence on the efficacy of lactose metabolites as FIBs of milk intake. Due to the large proportion of lactase-persistent compared to lactase non-persistent individuals (104 *vs.* 6), which is partly expected in the Dutch population, the effects of lactase persistence on the efficacy of lactose metabolites as FIBs of milk intake could not be evaluated in this study with sufficient statistical power. However, in studies involving larger populations or comprising different ethnic populations, the presence of these genetic variants may be magnified, which could affect the accuracy of FIBs. Beyond genetic factors, similar studies could be conducted to explore the effects of sex, age, body type (*e.g.*, obese vs. non-obese), or even gut microbiota differences that could affect the identification and validation of potential FIBs. Human variability also contributes to inconsistencies in diet-health associations (78). Participants consuming the same foods can have variable responses to nutrients, foods, or diets. This was shown in a landmark study by Zeevi *et al.* (79), where high inter-individual variability was observed in postprandial blood glucose response between participants consuming identical meals. Thus, further understanding the interplay between FIBs and inter-individual variability in metabolism can be useful for future personalized nutrition initiatives and tailored dietary advice.

Understanding the contribution of fermented foods to sustainable diets: fermentation as 'Agriculture 2.0'

Finally, it would be remiss not to think more broadly of food fermentation as a versatile tool to help extend the world's food supply. Global food and agricultural production accounts for a quarter of the world's greenhouse gas emissions, and the global population is poised to reach 10 billion by 2050 (80, 81). With so many mouths to feed, there is a pressing need to reflect on how to improve the sustainability of human diets to best protect the natural environment. A recent report from the EAT-Lancet Commission advised consumers to eat more plant-based foods and less animal-based foods in order to relieve the human footprint on food systems (82). Fermented foods could play an important role in achieving healthy and sustainable plant-based diets. For millennia, food fermentation has been used as a strategy to avoid food waste by increasing the shelf life of fresh foods. In the modern food supply chain, fermentation of plant-based foods can be an effective strategy to improve their shelf life, storage, and transport (83). The consumption of many protein-rich vegetable or legume alternatives to meat are constrained by the high presence of anti-nutrients, which can be reduced by food fermentation. In addition, fermentation can help increase the palatability of plant-based foods by introducing new flavours and reducing unpleasant flavours. On the other hand, fermentation may be used as a strategy to reduce meat consumption (rather than replacing it completely), through the development of novel fermented food products with mixed protein and animal sources (84). While it is known that fermentation of foods can also increase their nutrient density (thus reducing the need to consume large amounts of foods), industrial fermentations also require a lot of energy. Thus, more work is required to elucidate the nutritional and sustainable trade-offs of fermented food production and consumption.

Concluding remarks

The research presented in this thesis advances our understanding of the types and levels of fermented foods and dairy foods consumed in the Dutch adult diet. The suitability of single and combined candidate FIBs of fermented foods and dairy foods were explored in a comprehensive systematic review of the literature, as well as in targeted and non-targeted analyses of the plasma and urine metabolomes of free-living Dutch adults. Overall, the results suggest that combined FIBs may capture food intakes with greater accuracy over single FIBs. Further elucidation of the best combinations of FIBs would be beneficial in improving the dietary intake assessment of fermented foods and dairy foods in future studies in order to provide more reliable approximations of diet-health relationships. In addition, associations conducted between self-reported fermented food and dairy food intake, candidate FIBs, and CMD risk parameters suggests that FIBs may also reveal the (cardio)metabolic effect of food intake. However, the relatively weak and inconclusive associations need to be further explored and verified in larger studies. Further research into the nutritional qualities of fermented foods could help develop fermented food products with beneficial properties, and better facilitate public health guidance for inclusion of these foods in the diet to promote (cardiometabolic) health.

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SUMMARY

Cardiometabolic diseases (CMDs) represent one of the largest health and socioeconomic burdens to modern society. Fermented foods are commonly consumed in diets worldwide and can be an important part of an effective dietary strategy to help prevent and manage CMDs. However, precise associations between the consumption of fermented foods and cardiometabolic health have not been well-established. This could be partly due to the difficulty of accurately capturing their intake through self-report dietary assessment methods. This thesis set out to identify food intake biomarkers (FIBs) for the habitual intake fermented foods, which can act as more objective measures of intake. In addition, several candidate FIBs for dairy foods that were identified from non-targeted, controlled intervention studies needed to be validated under free-living conditions. These aims were achieved using data from a free-living cohort in the Netherlands (NQplus). Alongside, associations between fermented food and dairy (food) intake, biomarkers, and cardiometabolic factors were explored, which can reveal insights about the diverse metabolic effects of these foods.

Firstly, to capture existing FIBs that have been identified for various fermented foods consumed globally, a systematic literature review was conducted and the results presented in **Chapter 2**. The majority of FIBs captured in the literature review were those identified for the intake of coffee, wine, cocoa, beer, and bread. Many of the candidate FIBs identified were found to be non-specific. Thus, greater specificity may be observed with a combination of compounds identified for individual fermented foods, food groups, and from fermentation processes.

In **Chapter 3**, the types and levels of fermented foods consumed in the Dutch adult diet was determined from analyzing food lists from food frequency questionnaires (FFQs) and 24-h recalls. Approximately 16-18% of foods consumed by Dutch adults consisted of fermented food items. Fermented foods with the highest consumption (on a g/day basis) included coffee, yoghurts, beer, wholegrain bread, wine, and cheese. A relative validation of the FFQ (compared to multiple 24-h recalls) for estimating the intakes of fermented foods was also peformed. Acceptable or good validity was achieved for commonly consumed foods in The Netherlands, including fermented beverages (coffee), wholegrain and rye bread, and fermented dairy (cheeses), but not for less frequently consumed foods, such as quark and buttermilk. Alongside revealing the strengths of the FFQ in providing reliable intake estimates for various fermented foods, this analysis also revealed the limitations of the FFQ, in that a fairly narrow range of fermented foods were able to be assessed.

In **Chapter 4**, the robustness of several previously-identified FIBs of dairy foods (milk, cheese, and yoghurt) were evaluated in the NQplus cohort using both single and multi-marker approaches. Multi-marker models that also accounted for common physiological covariates better captured the subtle differences for milk and cheese intake over single-marker models. However, no significant single or multi-marker models were able to properly assess yoghurt intake. The modest associations observed suggests that the multi-marker panels could be improved with the inclusion of other (novel) FIBs. Alongside, further examination of other facets of validity of these biomarkers is warranted.

The analysis on dairy was further expanded in **Chapter 5**, where a panel of 67 milk-derived free fatty acids were explored as both biomarkers of dairy intake and of its metabolic effect. Fourteen free fatty acids in plasma were positively associated with dairy intake (and in particular, dairy fat inake). Additionally, several of these fatty acids were also associated with plasma total and low-density lipoprotein cholesterol, blood pressure, and a composite score of cardiovascular risk. These findings suggest that milk-derived fatty acids could act as biomarkers for dairy intake and metabolic effect linked to CMD. However, no clear associations were observed between dairy intake and CMD risk factors.

Finally, in **Chapter 6**, a non-targeted identification of FIBs for fermented foods was conducted. Out of the 36 metabolites identified in the plasma and urine metabolomes of NQplus participants, the majority corresponded to the intakes of coffee, wine, and beer (none were identified for cocoa, bread, cheese, or yoghurt intake). Some metabolites appeared to originate from the food raw material and have been previously linked with a specific food (*e.g.,* trigonelline for coffee), while others overlapped across several fermented foods (e.g., ethyl α-D-glucopyranoside for both wine and beer). The identification of these FIBs in a free-living cohort could be an indicator of their robustness. In addition, several possible fermentation-dependent metabolites were identified (*e.g.,* erythritol and citramalate). Associations between these identified metabolites with CMD risk parameters were weak and inconclusive. Thus, further evaluation in a larger cohort is required in order to confirm their relationship with CMD risk.

Collectively, the results from these chapters indicate that there is potential in further exploring multi-markers of fermented food intake. Regarding the effects of FIBs of fermented foods and dairy foods on cardiometabolic health, more studies are required in larger populations with a larger gradient of CMD risk.

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我非常想念你们.

To the most important human in my life, **Christoph.** What a whirlwind the past few years have been! First in Colorado, then Canada, then back and forth from the Netherlands/Switzerland to Norway, and now in Germany. We always knew that long distance would be temporary and that we would find a place for us in this world. Thank you for always giving me your attention, making me laugh, and forcing me to stop working and relax. Thank you for helping me with my loops when I got stuck (I knew I got an engineer for a reason). But most of all, thank you for all you have sacrificed so I could pursue my dream. You light up my life, and I'm so happy we were able to finally get married and bring Pi into our lives. Looking forward to what life brings for us next!

And now to the most important non-human in my life, **Pi**. Thank you for forcing me to walk with you twice a day (my back is also thankful). You have an uncanny ability of introducing yourself in all my online meetings at the worst possible times. Even though you often make me jealous (e.g., when I am hard at work and I look over to see you napping), and sometimes wake me up at an ungodly hour, seeing your little face and wagging tail makes all my worries go away.

ABOUT THE AUTHOR

Curriculum Vitae

Katherine Jia Li was born on September 6, 1990 in Zhengzhou, China, and grew up in Australia and Canada. She obtained her secondary school diploma from Glenview Park Secondary School in Cambridge, Ontario, Canada, and received the Governor General's Academic Medal for having the highest overall academic standing upon graduation. In 2008, she started an Honors Bachelor of Science at the University of Toronto, double-majoring in pharmacology and immunology. She completed short research projects in pharmacology and toxicology at the Hospital for Sick Children and with the Canadian Harm Reduction Agency. This was followed by a summer project and a fourth year bachelor's thesis in the Department of Chemical Engineering with Dr. Alison McGuigan, where she helped to design an assay to measure cell mixing for potential drug screening applications. While at the University of Toronto, Katherine was a Residence Don at Woodsworth College, a Social and Behavioural Programs Assistant at the Sustainability office, and served on a number of student groups. These activities led to Katherine receiving the coveted Gordon Cressy Student Leadership Award upon graduation. In 2013, she started as a toxicologist in the Food and Nutrition Group at Intertek Scientific & Regulatory Consultancy (formerly Cantox) in Mississauga, Canada. After working for a few years, she pursued a Masters of Science in toxicology at Colorado State University, in Fort Collins, USA. She joined the Nutrition and Toxicology laboratory led by Dr. Elizabeth Ryan to combine her toxicology background with a growing interest in food and nutrition research. Her Master's thesis, entitled "Plasma metabolome of children with aberrant cholesterol and modulation by navy bean and rice bran consumption", received 3rd place in the nutrition division poster competition at the Institute of Food Technologists (IFT) 2018 annual meeting. In late 2018, Katherine started as a doctoral candidate on the Cardioferment project under the supervision of Prof. Edith Feskens and Dr. Guy Vergères, and performed research at both Wageningen University & Research in Wageningen, The Netherlands and Agroscope in Bern, Switzerland. Alongside her PhD project, Katherine has helped to write several grant proposals, including a Marie-Curie ITN and for the Periodic Table of Food Initiative. In late 2021, Katherine started as a researcher on the FLOW project alongside a team of collaborative researchers from Wageningen University, University of Twente, and Eindhoven University of Technology. Katherine is currently conducting and coordinating various research projects at Wageningen University and Agroscope.

List of Publications

Published articles

1. **Li KJ,** Burton-Pimentel KJ, Brouwer-Brolsma EM, Feskens EJM, Blaser C, Badertscher R, Portmann R, Vergères G. (2021). Evaluating the robustness of biomarkers of dairy food intake in a free-living population using single- and multi-marker approaches. *Metabolites*. 11(6), 395. https://doi.org/10.3390/metabo11060395.
2. **Li KJ,** Brouwer-Brolsma EM, Burton-Pimentel KJ, Vergères G, Feskens EJM. (2021). A systematic review to identify biomarkers of intake for fermented food products. *Genes Nutr*. 16(1):5. doi: 10.1186/s12263-021-00686-4.
3. Koedam E, **Li KJ,** Burton KJ. (2020). Place des produits laitiers fermentés dans le traitement des maladies cardio-vasculaires. *Pratiques en Nutrition.* 6852(61):1-47. doi: 10.1016/j.pranut.2020.06.008.
4. **Li KJ**, Brouwer-Brolsma EM, Burton-Pimentel KJ, Vergères G, Feskens EJM. (2020). Prevalence of fermented foods in the Dutch adult diet and validation of a food frequency questionnaire for estimating their intake in the NQplus cohort. *BMC Nutr.* 6(1):69. doi: 10.1186/s40795-020-00394-z.
5. **Li KJ,** Puccetti-Jenkins N, Luckasen G, Rao S, Ryan EP. (2018). Plasma Metabolomics of Children with Aberrant Serum Lipids and Inadequate Micronutrient Intake. *PLoS One*. Oct 31;13(10):e0205899.
6. **Li KJ**, Borresen EC, Puccetti-Jenkins N, Luckasen G, Ryan EP. (2018). Navy Bean and Rice Bran Intake Alters the Plasma Metabolome of Children at Risk for Cardiovascular Disease. *Front. Nutr.* 4:71. doi: 10.3389/fnut.2017.00071.
7. Paulionis L, Walters B, and **Li KJ.** (2015). Authorised EU Health Claims on Pectins (Chapter 9). In: Sadler MJ, editor. Foods, Nutrients and Food Ingredients with Authorised EU Health Claims: Volume 2. (Woodhead Publishing Series in Food Science, Technology and Nutrition, number 286). Amsterdam, The Netherlands: Elsevier/Cambridge, UK: Woodhead Publishing Limited, pp. 153-174.
8. Javaherian S*, **Li KJ***, McGuigan A. (2013). A simple and rapid method for generating patterned co-cultures with stable interfaces. *Biotechniques*. 55(1):21-6. doi: 10.2144/000114051.
   * Co-first author.

Expected articles

1. **Li KJ**, Burton-Pimentel KJ, Brouwer-Brolsma EM, Vergères G, Feskens EJM. How can new personalized nutrition tools improve health? (In press – *Front. Young Minds*)
2. **Li KJ,** Brouwer-Brolsma EM, Fleuti C, Badertscher R, Vergères G, Feskens EJM, Burton-Pimentel KJ. Associations between dairy fat intake, milk-derived free fatty acids, and cardiometabolic risk in Dutch adults. (Submitted)
3. Baxter BA, **Li KJ**, Zarei I, Zheng L, Wolfe LM, Rao S, Ryan EP. Non-targeted and targeted metabolomics reveals dietary exposure biomarkers for navy bean and rice bran consumption in children and adults. (Submitted)
4. **Li KJ,** Burton-Pimentel KJ, Brouwer-Brolsma EM, Blaser C, Badertscher R, Pimentel G, Portmann R, Feskens EJM, Vergères G. Identifying plasma and urinary biomarkers of fermented food intake and their associations with cardiometabolic health in a Dutch observational cohort. (In preparation)
5. **Li KJ**, Lasschuijt M, Brouwer-Brolsma EM. A systematic review of methods and technologies used to measure breastmilk intake, breastfeeding behaviour, and breast physiology. (In preparation)
6. Mevissen SJ, Pijnappel MC, Haarman JAM, Lasschuijt M, Zhang X, Kalinauskaite I, **Li KJ**, de Gooijer F, Brouwer-Brolsma EM. FLOW: measures of breastfeeding and infant sucking behaviour. (In preparation)

List of Presentations

Oral presentations

- *Evaluating the robustness of biomarkers of dairy food intake in a free-living population using single- and multi-marker approaches.* Agroscope PhD/Postdoc Symposium. Online (2021)
- *Non-targeted and targeted metabolomics to identify and validate biomarkers of fermented dairy intake.* Symposium – Biomarkers for food and beverage intake – results from the FoodBAll project. eICDAM (2021)
- *Capturing fermented food intake in a free-living population: challenges and opportunities.* Colloquium – Adding value to food by fermentation. Agroscope, Bern, Switzerland (2020)
- *Non-Targeted and Targeted Metabolomics to Identify and Validate Biomarkers of Fermented Food Intake in a Dutch Observational Cohort.* Swiss Metabolomics Society Annual Meeting. Inselspital Bern, Switzerland (2019)
- *Identifying and Validating Biomarkers of Fermented Food Intake to Better Understand Their Associations with Cardiometabolic Health.* NuGO Week. Bern, Switzerland (2019)
- *Plasma metabolome of children with elevated cholesterol and modulation by navy bean and rice bran consumption.* 19th Annual CVMBS Research Day. Fort Collins, CO (2018)
- *Non-Targeted Plasma Metabolic Profiling of Children with Elevated Cholesterol Consuming Navy Bean and Rice Bran: A Randomized Controlled Trial*. 18th Annual CVMBS Research Day. Fort Collins, CO (2017)

Poster presentations

- **Li KJ**, Brouwer-Brolsma EM, Fleuti C, Spahni M, Badertscher R, Feskens EJM, Vergères G, Burton-Pimentel KJ. *Dairy and cardiometabolic health: examining the dual role of milk-derived fatty acids as biomarkers of intake and effect in a free-living cohort.* WEON (2021)
- **Li KJ,** Brouwer-Brolsma EM, Burton-Pimentel KJ, Vergères G, Feskens EJM. *Prevalence of fermented foods in the Dutch adult diet and validation of a food frequency questionnaire for estimating their intake in the NQplus cohort.* eICDAM (2021)
- **Li KJ**, Burton-Pimentel KJ, Brouwer-Brolsma EM, Feskens EJM, Blaser C, Badertscher R, Portmann R, Vergères G. *Validating Biomarkers of dairy food intake in a free-living observational cohort.* 16th Annual Conference of the Metabolomics Society (2020)
- Ryan EP, Baxter B, **Li KJ,** Wolfe L, Yao L, Broeckling C, Borresen E, Zhang L, Zarei I, Beale M, Rao S, Smith H, Zambrana L, Koita O, Vilchez S. *Developing Biomarkers of Rice Bran and Navy Bean Intake via Integrated Metabolomics from Infants, Children and Adults for Association with Gut Health Properties.* Curr. Dev. Nutr. 4(S2), 463. https://doi.org/10.1093/cdn/nzaa045_096 (2020)
- Koedam E, **Li KJ**, Brouwer-Brolsma EM, Badertscher R, Spahni M, Vergères G, Feskens EM, Burton KJ. *Associating Circulating Lipid Biomarkers of Fermented Dairy Food Intake with Markers of Cardiometabolic Health.* Swiss Metabolomics Society Annual Meeting. Inselspital Bern, Switzerland (2019)
- **Li KJ**, Brouwer-Brolsma EM, Vergères G, Feskens EM, Burton KJ. Agroscope PhD/Post-Doc Symposium. *Associating Markers of Intake of Fermented Foods with Cardiometabolic Factors in a Real-Life Observational Cohort: The Cardioferment Project.* Agroscope PhD/Postdoc Symposium. Zurich, Switzerland (2018)
- **Li KJ**, Zarei I, Oppel R, Wolfe L, Ryan EP. *Developing Dietary Biomarkers of Navy Bean and Rice Bran Intake in the Plasma Metabolome of Children and Adults with Chronic Disease Risk Factors.* Institute of Food Technologists (IFT) Meeting. Chicago, IL (2018)
- **Li KJ**, Jenkins-Puccetti N, Luckasen G, Rao S, Ryan EP. *Plasma Metabolome of Children with Elevated Cholesterol and Cardiovascular Risk.* Graduate Student Showcase. Fort Collins, CO (2017)
- Oppel R, Zarei I, **Li KJ**, Borresen E, Brown R, Jenkins-Puccetti N, Luckasen G, Ryan EP. *Rice Bran and Bean Metabolomes for Human Dietary Exposure Biomarkers.* USDA Project Director's Meeting. Institute of Food Technologists (IFT) Meeting. Las Vegas, NV (2017)
- **Li KJ**, Borresen EC., Puccetti-Jenkins N, Luckasen G, Ryan EP. *Non-Targeted Plasma Metabolic Profiling of Children with Elevated Cholesterol Consuming Navy Bean and Rice Bran: A Randomized*

Controlled Trial. Colorado Biological Mass Spectrometry Society Spring 2017 Meeting. University of Colorado Anschutz Medical Campus. Denver, CO (2017)

- **Li KJ**, Borresen EC., Puccetti-Jenkins N, Luckasen G, Ryan EP. *Non-Targeted Plasma Metabolic Profiling of Children with Elevated Cholesterol Consuming Navy Bean and Rice Bran: A Randomized Controlled Trial.* 56th Annual Meeting of the Society of Toxicology. Baltimore, MD (2017)
- **Li KJ**, Borresen EC., Puccetti-Jenkins N, Luckasen G, Ryan EP. *Non-Targeted Plasma Metabolic Profiling of Children with Elevated Cholesterol Consuming Navy Bean and Rice Bran: A Randomized Controlled Trial*. CSU Center for Environmental Medicine Symposium. Fort Collins, CO (2017)
- **Li KJ**, Paz-Mejia A, McGuigan A. *Investigating the Polarization of MDCK Epithelial Cells on Glass*. Undergraduate Engineering Research Day, Bahen Centre for Information Technology, University of Toronto (2011)

Invited lectures

- *Acrylamide: Toxicology & Current Measurement Methods.* AGEXPORT Alimentos y Bebidas. Guatemala City, Guatemala (2017)
- *Acrylamide: Toxicology, Regulations, & Measurement Methods.* Universidad de San Carlos de Guatemala and Universidad de Valle de Guatemala. Guatemala City, Guatemala (2017)
- *Food Safety Risk Assessment.* Guest lecture for graduate course in environmental health policy (PBHL 530). Colorado State University (2017)
- *Translating Science*. Guest presentation for undergraduate course in pharmacokinetics (PCL201). University of Toronto (2013)
- *Reducing Harms Associated with the Stimulant Use of Wellbutrin*. Metropolitan Hall, Toronto, ON (2013)

Overview of completed training activities

Discipline specific activities		
	Organizer (location)	**Year**
Agroscope PhD/PostDoc Symposium	Agroscope (Bern, CH)	2019
NuGO Nutritional Metabolomics pre-conference course	NuGO, (Bern, CH)	2019
NuGO conference	NuGO, (Bern, CH)	2019
Hands-on data analysis for Metabolic Profiling	Imperial College London (London, UK)	2020
Agroscope Fermentomics Colloquium	Agroscope (Bern, CH)	2020
Swiss Metabolomics Society conference	Swiss Metabolomics Society (Bern, CH)	2020
Metabolomics Society annual meeting	Metabolomics Society (online)	2021
ICDAM conference + oral presentation	WUR (online)	2021
Agroscope PhD/PostDoc Symposium	Agroscope (online)	2021
WEON conference + pre-conference course	VuA (online)	2021
General courses		
Name of the course	**Organizer (location)**	**Year**
PhD Competence assessment	WGS (Wageningen, NL)	2018
VLAG PhD Week	VLAG (Baarlo, NL)	2019
Erasmus MC Basic Course on 'R'	Erasmus MC (Rotterdam, NL)	2018
RadboudUMC Project Supervision courses	RadboudUMC (online)	2020
Intensive writing week	WUR/in'to Languages (online)	2021
Researcher management and leadership Training	Coursera/University of Colorado system (online)	2021
Data science visualization	Coursera/Harvard (online)	2021
Other activities		
Name of the course	**Organizer (location)**	**Year**
Preparation of research proposal	WUR/HNE-GN (Wageningen, NL)	2018
Dept. meetings and seminars at WUR	WUR/HNE (Wageningen, NL)	2018-21
Dept. meetings and seminars at Agroscope	Agroscope (Bern, CH and online)	2018-21
Rothman lunch lectures: Introduction to Epidemiology	WUR (online)	2021

Colophon

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