PRECISION NUTRITION

FOR CARDIOMETABOLIC HEALTH

INSIGHTS FROM HUMAN DIETARY INTERVENTION STUDIES

Anouk Gijbels

Propositions

1. Refining metabolic phenotyping with blood lipid measures in addition to tissue-specific insulin resistance will enhance health benefits of precision nutrition. (this thesis)

2. At least 10 more years of research are required before precision nutrition based on metabolic phenotyping is ready for implementation in practice. (this thesis)

3. The most fundamental question in the context of nutritional epidemiology is “Compared to what?”.

4. Curiosity fuels science in the long term, but slows it down in the short term.

5. Hyper-competition for research funding hinders scientific progress.

6. The key target organ for reversal of the current obesity and metabolic health crises is the legislative organ of government.

7. The sense of perspective is as invaluable as the five basic senses for navigating the world.

Propositions belonging to the thesis, entitled

Precision nutrition for cardiometabolic health: insights from human dietary intervention studies

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Wageningen, 23 June 2023

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This research was conducted under the auspices of VLAG Graduate School (Biobased, Biomolecular, Chemical, Food, and Nutrition sciences).

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 23 June 2023
at 11 a.m. in the Omnia Auditorium

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Precision nutrition for cardiometabolic health: insights from human dietary intervention studies

334 pages

PhD thesis, Wageningen University, Wageningen, the Netherlands (2023)
With references, with summary in English

ISBN 978-94-6447-656-9
DOI https://doi.org/10.18174/591161

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General introduction

Suboptimal diet: a growing driver of disease burden

In the past three decades, improvements in health have lagged behind the rise in life expectancy; people live longer, but those years are increasingly spent in poor health.[1] A growing proportion of this disease burden is attributable to cardiometabolic abnormalities, including high blood pressure, excess body weight, and elevated plasma glucose and cholesterol levels.[2,3] Suboptimal diet is the most important modifiable risk factor for these metabolic impairments; globally, one in five deaths is estimated to be attributable to poor diet.[4] Improving diet quality thus is an important strategy for promoting metabolic health. There is general consensus on the foods that define a healthy diet,[5] but the diet composition most optimal for metabolic health may differ between individuals. In recent years, nutrition research has demonstrated great inter-individual variation in how people respond to foods, meals, or diet. Emerging evidence indicates that metabolic heterogeneity is partly responsible for those differential responses. The current one-size-fits-all dietary guidelines may thus require fine-tuning according to an individual's metabolic phenotype.

The ever-increasing burden of metabolic disorders calls for effective intervention strategies that ameliorate metabolic dysfunction and prevent progression to overt cardiometabolic disease. Improving diet quality is a well-established method for promoting metabolic health, but the mechanisms by which specific diets or nutrients affect metabolic parameters are poorly understood. The research in this thesis aimed to contribute to a better understanding of the role of metabolic heterogeneity in response to diet, with a specific focus on tissue-specific insulin resistance. In addition, we aimed to contribute to a better understanding of the mechanisms by which diet affects metabolic health by investigating circulating metabolites that are related to cardiometabolic health and liver health or function.

Diet-related metabolic dysfunction

To maintain metabolic homeostasis in varying circumstances, humans have evolved an intricate system to store nutrients during feeding and mobilise stored nutrients during fasting. The key organs orchestrating these processes are the liver, adipose tissue, skeletal muscle, pancreas, and gut. While it made us very well-adapted to times of food scarcity, the chronic energy and nutrient oversupply of modern times puts a major strain on this system and disrupts various metabolic processes in these key organs.[6] Such metabolic perturbations can result in glucose intolerance, insulin resistance, dyslipidemia, ectopic fat accumulation, and low-grade systemic inflammation, which in turn increase the risk of cardiometabolic diseases such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).[7,8] It is now well-established that

there is great heterogeneity in the development of cardiometabolic disease. Not only does the aetiology of metabolic abnormalities differ between individuals, so do the trajectories towards overt cardiometabolic disease.[9-15] A better understanding of heterogeneity in metabolic dysfunction may provide leads for intervention strategies that more successfully target those abnormalities.

Adiposity

Ever since obesity was recognised as a major health problem in high-income countries in the 1970s,[16] and as a global epidemic by the World Health Organization in 1997,[17] its prevalence has continued to increase. Prevalence rates have tripled since 1975, with more than one-third of the worldwide adult population and about two-thirds of adults in high-income countries having overweight or obesity in 2016. While excess total body fat mediates many of the metabolic complications observed in obesity, adipose tissue quantity per se is not the best predictor of cardiometabolic risk. One in three adults with obesity is estimated to be metabolically healthy, whereas as many as 24% of normal-weight adults and 55% of normal-weight adults aged 65 years or older are reported to have an adverse metabolic profile.[18]

Rather than the quantity, the anatomic location and function of adipose tissue largely determine its metabolic effects. Adipose tissue accumulation in the upper-body or abdominal region – commonly termed abdominal or central obesity - is closely associated with metabolic abnormalities. In contrast, fat accumulation in the lower-body or gluteofemoral region may confer a protective effect.[19,20] Dysfunctional adipose tissue is characterised by an impaired capacity to store lipids subcutaneously and a reduced suppression of lipolysis in response to insulin.[21] The consequential systemic lipid overflow promotes fat accumulation in the visceral adipose tissue and non-adipose tissues such as the liver, skeletal muscle, and pancreas, which in turn may induce insulin resistance.[22]

Glucose metabolism and insulin resistance

In healthy individuals, plasma glucose levels are maintained within the normal range under the coordinated control of insulin. In the fasting state, plasma glucose is almost exclusively derived from hepatic glycogen breakdown and hepatic gluconeogenesis, i.e. glucose synthesis from non-carbohydrate precursors, including lactate, amino acids, and glycerol. After a meal, the subsequent rise in plasma glucose concentrations promotes insulin secretion from the pancreatic β-cells. Insulin orchestrates a switch from glucose production to glucose utilisation by suppressing endogenous glucose production in the liver and stimulating glucose uptake in the liver, skeletal muscle, and adipose tissue for storage or use.[23] Adipose tissue quantitively only accounts for a minor portion of insulin-stimulated glucose disposal. Insulin's main action in adipose tissue is suppressing lipolysis, thereby reducing fatty acid and glycerol supply to the liver and indirectly suppressing hepatic glucose production.[23]

Adiposity is frequently accompanied by insulin resistance, which is impaired sensitivity of insulin's target tissues skeletal muscle, liver, and adipose tissue to insulin.[7,8] Reduced insulin sensitivity can initially be compensated for by increased pancreatic insulin secretion and decreased hepatic insulin clearance, resulting in hyperinsulinemia, thereby maintaining blood glucose levels within the normal range. However, with progressive worsening of insulin resistance and β-cell function, the β-cells eventually fail to sustain sufficient insulin secretion for proper glycemic control, which can result in prediabetes and, ultimately, type 2 diabetes mellitus (T2DM).

The pathogenesis of insulin resistance is complex and likely involves many mechanisms, including inflammation, lipotoxicity, oxidative stress, and mitochondrial dysfunction.[23] Adipose tissue dysfunction promotes lipid accumulation in the liver and skeletal muscle. While most of these lipids are stored as relatively inert TAGs, other lipid species, including diacylglycerols (DAG) and ceramides, may also accumulate. These lipids - particularly DAGs and possibly ceramides - can impair local insulin signalling and may thereby induce insulin resistance in these tissues.[24-26] The resulting hyperglycemia and hyperinsulinemia further promote liver fat accumulation by inducing hepatic *de novo* lipogenesis by activating the transcription factors carbohydrate response element binding protein (ChREBP) and sterol regulatory element-binding protein 1c (SREBP1c), respectively.[27,28] The mechanisms underlying adipose tissue insulin resistance are less well-studied and likely comprise a multitude of cellular stressors including low-grade inflammation.[23] Impaired insulin-mediated suppression of lipolysis in adipose tissue promotes fatty acid and glycerol flux to the liver and peripheral tissues, thereby stimulating hepatic gluconeogenesis and ectopic lipid accumulation.[21,29]

Tissue-specific insulin resistance

Although insulin resistance in the various target tissues often develops simultaneously, recent studies suggest that there may be inter-individual differences in the rate and severity of development between the different tissues.[30-33] This implies that some individuals may be predominantly resistant to insulin in skeletal muscle, while others may have primary insulin resistance in the liver. Skeletal muscle is the primary site for postprandial glucose uptake, and hence, insulin resistance in the skeletal muscle results in elevated postprandial glucose levels due to impaired glycogenesis.[34] In the liver, insulin resistance disrupts the coordinated insulin-mediated suppression of gluconeogenesis and glycogen breakdown, and the stimulation of glycogen synthesis, which primarily results in increased hepatic glucose output during fasting and the early postprandial phase.[35] As described before, adipose tissue insulin resistance also contributes to elevated glucose levels by promoting hepatic gluconeogenesis via increased fatty acid delivery to the peripheral tissues from lipolysis.[29]

Besides differences in the primary site of impaired insulin action and glucose homeostasis, tissue-specific insulin resistance has been associated with distinct metabolic disturbances. Insulin resistance in the liver has been associated with visceral adiposity, elevated liver fat (Trouwborst et al., in preparation), and abnormalities in blood metabolome,[36-38] while insulin resistance in muscle has been associated with systemic and adipose tissue inflammation,[39] as well as increased muscle fat infiltration (Trouwborst et al., in preparation). Further characterisation of the (distinct) metabolic disturbances in tissue-specific insulin resistance can help to tailor interventions to ameliorate insulin resistance and prevent cardiometabolic disease.

Hepatic lipid metabolism

Diet- or obesity-related metabolic dysregulation is not only manifested by impaired glucose metabolism, but also by abnormalities in lipid metabolism. The liver is the master regulator of lipid metabolism. After the consumption of a fat-rich meal, dietary fat is hydrolysed in the small intestine and absorbed in the enterocytes.[40] Here, most of these lipids are packaged into chylomicron particles and released in the circulation via the lymphatic system, where they are transported to the peripheral tissues. Here, their lipids are hydrolysed by lipoprotein lipase (LpL) in adipose tissue and skeletal muscle for storage or oxidation, respectively. The remaining chylomicron remnants are cleared from the circulation by the liver and can be incorporated into very-low-density lipoproteins (VLDL).[41] Once in the circulation, VLDL delivers lipids to the peripheral tissues. VLDL remnants are either cleared by the liver or further metabolised into inter-mediate density lipoproteins (IDL) and ultimately low-density lipoprotein (LDL) particles as they become progressively depleted of TAGs.[41] LDL transports cholesterol to the peripheral tissues, and excess cholesterol is removed from the peripheral tissues and delivered to the liver by high-density lipoprotein (HDL) particles. After a meal, circulating VLDL-TAG concentrations increase before dietary fat appears in VLDL-TAG because VLDL-TAG and chylomicrons compete for hydrolysis by LpL.[42]

Insulin resistance is commonly accompanied by dyslipidemia, a blood lipid profile characterised by elevated TAG levels, low HDL, and a predominance of small, dense LDL particles. This pro-atherogenic profile is strongly linked to the development of CVD.[43,44] Postprandial hypertriglyceridemia has also been recognised as an important risk factor for CVD and related metabolic disorders, independent of fasting TAG or LDL cholesterol levels.[45-47] Hepatic and adipose tissue insulin resistance promote VLDL overproduction by increasing substrate availability via *de novo* lipogenesis and adipose tissue lipolysis, respectively.[48] As mentioned before, hyperglycemia and hyperinsulinemia also promote *de novo* lipogenesis, thereby contributing to elevated VLDL-TAG output. In addition, impaired hepatic lipid clearance and FA uptake into adipose tissue may contribute to elevated postprandial TAG.[49-51] When hepatic lipid synthesis and lipid supply exceed the liver's capacity for lipid removal by oxidation or secretion into VLDL, TAG can accumulate in the liver, which can ultimately result in steatosis or non-alcoholic fatty liver disease (NAFLD).[52] Hepatic steatosis has been closely associated to insulin resistance and dyslipidemia,[53] but the causal and temporal nature of these associations is unclear.

Fibroblast growth factor 21

The liver also mediates glucose and lipid metabolism by producing various hepatokines. The hepatokine fibroblast growth factor 21 (FGF21) has received major scientific interest in the past two decades. FGF21 is produced by the liver in response to a multitude of metabolic and cellular stressors. In rodents, FGF21 regulates metabolic homeostasis by promoting hepatic fatty acid oxidation and ketogenesis during fasting[54,55] and stimulating glucose disposal into adipose tissue during feeding[56]. Pharmacological treatment with FGF21 analogues has been shown to reduce plasma lipids and liver fat in individuals with T2DM, NAFLD, or obesity, but mostly without improvements in glucose metabolism, the latter in contrast to animal studies.[57-63] Paradoxical to the proposed functions of endogenous FGF21 and the favourable effects of pharmacological treatment, circulating FGF21 levels are typically elevated in conditions of metabolic dysregulation such as obesity, insulin resistance, NAFLD and T2DM.[64,65] FGF21 has also been implicated in the metabolic adaptations to both under- and overfeeding, with plasma FGF21 levels predicting the weight change in response to different hypo- or hypercaloric diets.[66-68] While FGF21's physiological role in humans remains largely elusive, circulating levels of FGF21 appear to be a marker of metabolic health status. Evidence from human studies, however, is still inconclusive.[69,70]

Early identification of metabolic dysfunction

Cardiometabolic disorders progress slowly, and it takes many years or even decades before clinical symptoms manifest. The traditional biomarkers routinely used in the clinic, such as fasting plasma glucose, cholesterol, and triglycerides, are intended for disease detection, and therefore are unsuitable for picking up more subtle metabolic perturbations. Extensive profiling of circulating metabolites, i.e. the use of metabolomics, may allow for the identification of early metabolic alterations. For example, by applying metabolomics, a fasting metabolite signature of branched-chain amino acids, aromatic amino acids, fatty acids, and lipoproteins has been identified that predicted the risk of T2DM up to 15 years before disease onset.[71,72] Measuring metabolites in response to a meal may be an even more sensitive measure. A meal provides a metabolic stressor to the body that requires a coordinated response from the gut, liver, pancreas, skeletal muscle, and adipose tissue to regain homeostasis. Hence, postprandial metabolite levels reflect the complex interplay between the key metabolic organs, and may provide more insights into the functioning of these organs than

circulating metabolites in the fasting state.[73] The detection of metabolic perturbations at an early stage - before the onset of overt metabolic disease - provides an opportunity for timely prevention of progression to cardiometabolic disease by lifestyle interventions such as dietary modification.

Dietary strategies to improve metabolic health

Restriction of caloric intake induces rapid and substantial improvements in cardio-metabolic health, with body weight reduction by as little as 5% already providing clinically meaningful health benefits, and larger weight reduction resulting in greater improvements.[74,75] Long-term weight loss maintenance, however, has proven difficult.[76] Lost weight is commonly regained, and related metabolic impairments return along with the weight regain. Some evidence even suggests that repeated cycles of weight loss followed by weight regain may be more detrimental to health than sustained overweight.[77] Hence, improving diet quality is an important strategy for ameliorating metabolic perturbations, also without weight loss. Overall, there is broad consensus on the main ingredients of a healthy dietary pattern: vegetables, fruits, whole grains, legumes, and nuts, and limited consumption of products rich in added sugars, refined carbohydrates, salt, or saturated fat such as sugar-sweetened beverages and processed meat.[5] Emerging evidence suggests that the diet most optimal for metabolic health may differ between individuals. We may thus need to move beyond the current one-size-fits-all dietary guidelines to maximise diet-induced health effects.

Inter-individual variation in dietary response

Several studies have demonstrated great heterogeneity in individuals' metabolic response to dietary modification or diet-induced weight loss.[78-83] Although differences in adherence may partly account for this heterogeneity, it is becoming increasingly evident that other factors are likely also involved. Next to lifestyle and environmental factors, various biological factors have been suggested to affect the response to diet, including genetic variation, gut microbiome composition, and parameters related to glucose homeostasis, such as plasma glucose and insulin levels or indices derived from those.[83-88] Knowledge about this inter-individual variation can be harnessed to tailor dietary interventions to sub-groups that share specific characteristics, i.e. precision nutrition, or tailor diet to the individual, i.e. personalised nutrition. More customised dietary advice may be more effective for improving health than generalised advice.

Precision nutrition

Generally, two main approaches have been used in precision nutrition research. The first approach aims to improve diet quality by promoting behaviour change, and hence uses personalisation for more optimal delivery of dietary advice or for promoting dietary adherence. The second approach aims to optimise health benefits by tailoring the diet to match someone's biology best, thus basing the personalisation on biological features.[89] Ultimately, an integrative approach combining biological, environmental, behavioural, and psychological characteristics for tailor-made nutritional advice may prove to be most effective for promoting health.

The Food4Me study[90,91] was the first RCT to test the effects of more personalised nutrition on behaviour change. Participants from seven European countries received personalised dietary advice based on current dietary intake, phenotypic information including plasma glucose, cholesterol, fatty acids, and vitamin D, and genotypic information on five single-nucleotide polymorphisms (SNPs). After six months of intervention, personalised dietary advice improved diet quality more than generalised advice, although findings on the added value of including genotypic information are inconsistent.[90,92,93] Another approach has been used by O'Donovan and colleagues,[94,95] who clustered individuals into three metabolic phenotypes – termed metabotypes – according to their fasting glucose, triglycerides, total cholesterol, and HDL cholesterol. Using a decision tree, automated dietary advice based on those metabotypes, in combination with anthropometric data and BMI, was delivered. This method has been shown to generate similar advice as a trained dietician. An RCT testing the effectiveness of such personalised dietary advice based on metabotype has recently been completed, and results are expected to be published soon.[96]

For precision nutrition approaches based on novel biological features, the majority of suggested phenotype-diet interactions are based on *post-hoc* re-analyses of completed dietary trials. Very few prospective trials have been performed to validate such findings as of yet. In the DIETFITS trial,[97] Gardner and colleagues investigated whether weight loss success on a low-carbohydrate or low-fat diet was dependent on baseline insulin secretion or genotype (3 SNPs related to carbohydrate and lipid metabolism). Although large inter-individual variability was observed in weight loss on both diets, this was unrelated to baseline insulin secretion or genotype. In the PREVENTOMICS study,[98] the effectiveness of a personalised dietary plan based on genetic and metabolomics information for metabolic health outcomes was tested against generalised dietary advice. Based on 35 SNPs and 51 blood and urine metabolites, individuals were clustered into one of five metabotypes that represented disturbances in physiological processes related to carbohydrate metabolism, gut microbiota, lipid metabolism, inflammation, or oxidative stress. Recently, in a 10-week RCT,[98] personalised dietary advice based on these metabotypes was not shown to result in cardiometabolic health improvements compared to generalised advice.

So while many observational studies suggest that individuals may respond differentially to a diet based on their metabolic phenotype, and hence, precision nutrition may confer superior health benefits than generalised advice, there is a lack of trial evidence to support these findings.

Given their metabolic differences, individuals with tissue-specific insulin resistance may also benefit from different dietary interventions. Indeed, this was suggested by the findings of a post-hoc analysis of the CORDIOPREV-DIAB study.[88] In this study, individuals with predominant muscle insulin resistance had greater improvements in the disposition index – a composite marker of insulin secretion and insulin sensitivity – on a Mediterranean diet rich in mono-unsaturated fat (MUFA), while individuals with predominant liver insulin resistance benefitted more from a diet low in fat and rich in complex carbohydrates. Other studies have also indicated that dietary fat quality may particularly affect skeletal muscle insulin sensitivity,[99,100] whereas high-fibre diets may specifically target liver insulin resistance by lowering liver fat.[53,101]

Personalised nutrition

Inter-individual variation in dietary response has also been demonstrated at the individual level to acute meals.[102-104] In their landmark study in 2015,[102] Zeevi and colleagues showed that individuals have highly variable postprandial glucose responses to identical foods and that differences in gut microbiome composition and clinical parameters could predict this variation. Their machine-learning algorithm that integrated these individual characteristics to predict individual glycemic responses outperformed prediction based solely on meal features (i.e. macronutrient composition), which was subsequently validated in a different population.[105,106] The clinical effects of this algorithm-based personalised dietary advice have been tested in two trials: a 6-month dietary intervention based on this algorithm resulted in greater reductions in postprandial glycemic response, HbA1c, and fasting triglycerides as compared to a Mediterranean diet in individuals with prediabetes,[107] but not to greater weight loss as compared to a low-fat diet in individuals with obesity[108]. Importantly, however, the 'personalised diet' was effectively a low-carbohydrate diet, so before any firm conclusions can be drawn on whether the personalisation component of the algorithm-based dietary advice has any added value for improving health, a trial comparing the 'personalised diet' to a control diet that is similar in key determinants of glycemic responses such as carbohydrate content is required.

More recently, inter-individual variation in postprandial responses has been further characterised in the PREDICT study.[104] In this study, postprandial glycemic responses, as well as postprandial triglyceride and C-peptide (as a proxy for insulin) responses, were measured after multiple standardised meals. Berry and colleagues demonstrated that postprandial triglyceride responses were even more variable than glycemic responses, and they showed that this inter-individual variation was largely explained by fasting serum lipid and glycemic markers and not by genetics. In contrast, the most important determinants of postprandial glycemic responses were meal composition, meal context, and genetics. A machine-learning model based on these and other features, including anthropometry, gut microbiota composition, clinical parameters, and blood metabolites, successfully predicted postprandial glucose, triglyceride, and C-peptide responses to real-life meals. However, the prediction of postprandial triglyceride and C-peptide was less accurate than the prediction of postprandial glucose responses. This was likely partly due to the limited number of test meals used to measure triglyceride and C-peptide responses because the currently available methods to measure these blood markers are burdensome and difficult to perform in free-living conditions, unlike ontinuous glucose monitoring (CGM) for measuring interstitial glucose. Personalised dietary advice based on this algorithm is commercially available but has yet to be validated against generalised advice in a clinical trial.

Aim and outline of this thesis

The aim of this thesis was to explore the potential of precision nutrition based on tissue-specific insulin resistance phenotype for improving cardiometabolic health. In addition, we aimed to explore potential leads towards mechanisms by which diet affects metabolic health by investigating circulating metabolites that are related to cardiometabolic health and liver health or function.

Our group previously demonstrated that a 12-week weight loss diet with 25% energy restriction and high nutrient quality resulted in greater weight loss and a more anti-atherogenic blood lipid profile than a diet with similar energy restriction but low nutrient quality.[87] In **Chapter 2**, we aimed to explore whether circulating FGF21 levels are a marker of diet-induced changes in metabolic health by investigating the effects of these dietary interventions on fasting and postprandial plasma FGF21 levels. Additionally, we explored correlations of (changes in) plasma FGF21 with markers of metabolic health and weight loss.

Moreover, our group previously demonstrated that a 12-week refined wheat intervention modestly increased liver fat (+1.5% percentage points) in overweight individuals with mildly elevated cholesterol levels, while whole-grain wheat did not affect liver fat content.[109] In **Chapter 3**, we studied the effects of these wheat interventions on plasma betaine, choline, acylcarnitines, bile acids, and signalling lipids to explore potential mechanisms that underlie the unfavourable effect of refined wheat or preventive effect of whole grain consumption on liver fat accumulation.

To explore the potential of precision nutrition based on insulin resistance (IR) phenotype, we - for the first time - prospectively studied in a randomised clinical trial

whether individuals with tissue-specific insulin resistance benefit from different diets. In this 12-week two-centre trial, we investigated the efficacy of modulation of dietary macronutrient composition according to muscle IR and liver IR phenotypes on various outcomes. We hypothesised that individuals with the muscle IR phenotype would benefit most from a diet rich in MUFA (HMUFA), and individuals with the liver IR phenotype from a diet low in fat and rich in protein and fibre (LFHP). Participants were extensively phenotyped before and after the intervention. In **Chapter 4**, we describe the rationale, study design, and preliminary screening results of this trial. In **Chapter 6**, we present the main intervention effects on glucose homeostasis, body composition, clinical cardiometabolic markers, and well-being.

In **Chapter 5**, we aimed to gain a better understanding of fasting and postprandial metabolism in tissue-specific insulin resistance. We further characterised these IR phenotypes by investigating fasting and postprandial metabolite profiles, including lipoproteins, apolipoproteins, cholesterol, triglycerides, ketone bodies, and amino acids in response to a high-fat mixed meal in individuals with predominant muscle or liver insulin resistance in a cross-sectional study.

In **Chapter 7**, we aimed to further characterise the effects of LFHP and HMUFA diets in tissue-specific IR and identify leads towards potential underlying mechanisms. To that end, we investigated the effects of the LFHP and HMUFA diets on the plasma metabolite profile in both the fasting state and in response to a high-fat mixed meal in individuals with tissue-specific IR.

Finally, in **Chapter 8**, we discuss the main results and conclusions of the research in this thesis and suggest directions for future research.

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Plasma FGF21 levels are not associated with weight loss or improvements in metabolic health markers upon 12 weeks of energy restriction: secondary analysis of an RCT

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Nutrients **2022**, *14*(23), 5061

Abstract

Recent studies suggest that circulating fibroblast growth factor 21 (FGF21) may be a marker of metabolic health status. We performed a secondary analysis of a 12-week randomized controlled trial to investigate the effects of two energy restriction (ER) diets on fasting and postprandial plasma FGF21 levels, as well as to explore correlations of plasma FGF21 with metabolic health markers, (macro)nutrient intake and sweet-taste preference. Abdominally obese subjects aged 40–70 years (n = 110) were randomized to one of two 25% ER diets (high-nutrient-quality diet or low-nutrient-quality diet) or a control group. Plasma FGF21 was measured in the fasting state and 120 min after a mixed meal. Both ER diets did not affect fasting or postprandial plasma FGF21 levels despite weight loss and accompanying health improvements. At baseline, the postprandial FGF21 response was inversely correlated to fasting plasma glucose (ρ = −0.24, p = 0.020) and insulin (ρ = −0.32, p = 0.001), HOMA-IR (ρ = −0.34, p = 0.001), visceral adipose tissue (ρ = −0.24, p = 0.046), and the liver enzyme aspartate aminotransferase (ρ = −0.23, p = 0.021). Diet-induced changes in these markers did not correlate to changes in plasma FGF21 levels upon intervention. Baseline higher habitual polysaccharide intake, but not mono- and disaccharide intake or sweet-taste preference, was related to lower fasting plasma FGF21 (p = 0.022). In conclusion, we found no clear evidence that fasting plasma FGF21 is a marker for metabolic health status. Circulating FGF21 dynamics in response to an acute nutritional challenge may reflect metabolic health status better than fasting levels.

Keywords: FGF21; fibroblast growth factor 21; overweight; obesity; caloric restriction; energy restriction; weight loss; dietary intervention; liver fat; metabolic health

1. Introduction

Fibroblast growth factor 21 (FGF21) is a peptide hormone that regulates metabolic homeostasis [1,2]. The exact biological functions and regulation of FGF21 vary widely between tissues and are not completely understood [1,2]. Circulating FGF21 levels are typically elevated in conditions of impaired metabolic health, such as obesity, non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2DM) [3,4]. Accordingly, circulating FGF21 levels have been reported to positively associate with BMI, body fat, liver fat content, blood pressure, insulin resistance (IR) and atherogenic lipid profiles [5,6], as well as predict incident metabolic syndrome and T2DM [7,8]. It is unclear whether and how weight loss and accompanying health improvements affect FGF21 levels, with some trials reporting reduced levels of circulating FGF21 upon diet-induced weight loss [9,10,11,12,13], and others reporting no effects on FGF21 [14,15,16,17,18,19,20].

Recently, FGF21 has been implicated in the metabolic adaptations to both under- and overfeeding. In the DIETFITS trial, fasting FGF21 level was associated with diet-induced weight loss, with higher FGF21 levels at baseline being associated with larger weight loss in response to a 12-month low-fat or low-carbohydrate diet [20,21]. Furthermore, in a 6-week trial, the change in circulating FGF21 upon energy restriction was associated with weight loss, with individuals with larger increases in fasting FGF21 achieving greater weight loss [22]. In addition, in a short-term trial, greater increases in fasting plasma FGF21 in response to a 1-day hypercaloric low-protein, high-fat diet were associated with larger weight loss in free-living conditions after 6 months [23]. Plasma FGF21 (response) thus seems to be related to the ability to lose weight.

Next to its metabolic effects, FGF21 also appears to play a role in the regulation of nutrient intake via central nervous system (CNS) signaling. Multiple large human genome-wide association studies (GWAS) have reported associations between FGF21 gene variants and carbohydrate, sugar, and alcohol consumption and sweet-taste preference [24,25,26,27]. Furthermore, higher fasting FGF21 levels have been associated with lower sweet-taste preference [26] and lower soda consumption [28]. Animal studies suggest that in response to carbohydrate and in particular sugar intake, FGF21 is induced in the liver, enters the circulation and subsequently acts as negative feedback signal via the liver-brain-axis to suppress further sweet-taste preference and carbohydrate intake [29,30,31]. As of yet, evidence from human studies on the link between plasma FGF21 and nutrient intake and taste preference is sparse.

We previously reported the effects of two 12-week energy restriction (ER) diets differing in nutrient quality in overweight and obese adults [32]. Both ER diets resulted in substantial weight loss and concomitant improvements in various health

parameters, including reduced liver fat, and decreased fasting glucose and insulin levels. In the present study, we investigated whether these diet-induced changes in body weight and related health outcomes were accompanied by changes in fasting and postprandial FGF21 levels. Additionally, we explored correlations of plasma FGF21 with markers of metabolic health, weight loss, habitual (macro)nutrient intake and sweet-taste preference.

2. Materials and Methods

The present study is a secondary analysis of a 12-week parallel randomized controlled trial (RCT) that was performed at Wageningen University, the Netherlands, in 2014. The aim of this trial was to investigate the effects of two different ER diets on weight loss and cardiometabolic health outcomes. Details have been described previously [32].

2.1. Participants and Study Design

The study population consisted of 110 women and men aged 40–70 years with abdominal obesity (BMI > 27 kg/m2 or waist circumference > 88 cm for women or >102 cm for men). Exclusion criteria were diagnosis of diabetes, heavy alcohol consumption, smoking, unstable body weight, diagnosis of long-term medical condition, and use of medication that is known to interfere with glucose or lipid metabolism.

Eligible subjects were randomly assigned to one of three intervention groups: a 25% ER high-nutrient-quality diet (n = 40), a 25% ER low-nutrient-quality diet (n = 40), or a control group (n = 30). At baseline and after 12 weeks of intervention, participants visited hospital Gelderse Vallei (Ede, the Netherlands) for an MRI scan and—on a separate day—visited Wageningen University after an overnight fast for measurements of general health and blood metabolites in the fasting state, as well as in response to a mixed meal.

2.2. Dietary Intervention

The dietary intervention strategy has been described in detail previously [32]. Briefly, both the ER diets were energy restricted by 25% of the estimated energy requirement of each participant. The high-nutrient-quality diet (HQ) was designed to improve metabolic health and contained an increased amount of soy protein, fiber, monounsaturated fat (MUFA), and omega-3 fatty acids. The low-nutrient-quality diet (LQ) contained an increased amount of saturated fat (SFA), animal protein, and fructose. The control group did not receive an intervention and was instructed to maintain their usual dietary habits. Participants in the ER groups received dietary advice and key food products. Adherence to the ER diets was assessed based on participants' food diaries, taking reported deviations from the dietary advice and leftover key food products into account [32].

2.3. General Health Measures

Systolic and diastolic blood pressure were measured automatically for 10 min with a 3-min interval using a DINAMAP PRO100. After an overnight fast, blood samples were collected from an intravenous cannula before and 30, 60, 120, 180, 240, and 360 min after consumption of a liquid mixed meal (3833 kJ; 76.3 g carbohydrates, 17.6 g protein, 60 g fat). To estimate IR, we used the homeostatic model assessment of insulin resistance (HOMA-IR), which is calculated as (fasting glucose [mmol/L] × fasting insulin [mU/L])/22.5 [33]. IR was defined as HOMA-IR > 2.5 [34]. Incremental area under the curves (iAUC) for glucose and insulin were calculated using the trapezoid method.

2.4. Plasma FGF21

Plasma FGF21 levels were measured in plasma samples from the fasting state and 120 min after consumption of the mixed meal using ELISA, according to manufacturer's instructions (FGF-21 Human ELISA Kit, ab125966, Abcam, Cambridge, UK). The inter- and intra-assay coefficients of variation were 12.4% and 5.9%, respectively. All samples from two subjects and one sample from another subject fell outside of the range of the standard curve and were therefore excluded from analyses.

2.5. Intra-Hepatic Lipid Content and Abdominal Fat Distribution

Intra-hepatic lipid content (IHL) was quantified using proton magnetic spectroscopy (^{1}H-MRS) on a 3T whole-body scanner (Siemens, Munich, Germany). Details have been described previously [32]. MRS spectra were analyzed using jMRUI software v5.2. NAFLD was defined as IHL > 5.56% [35]. Abdominal fat distribution was evaluated as subcutaneous (SAT) and visceral adipose tissue (VAT) areas in the abdomen and assessed using magnetic resonance imaging (MRI). SAT and VAT were quantified in a single-slice transverse image at the inter-vertebral space L3-L4 using the semi-automatic software program HippoFatTM [36].

2.6. Clinical Chemistry

Plasma glucose, insulin, and triglycerides, as well as serum total cholesterol, HDL cholesterol and safety parameters of liver function (aminotransferase [ALAT], aspartate aminotransferase [ASAT], and gamma-glutamyl transferase [γGT]) were analyzed photometrically (Cobas 8000, Roche Diagnostic Limited, Switzerland) by a center for medical diagnostics (Stichting Huisartsenlaboratorium Oost, Velp, the Netherlands). Plasma free fatty acids (FFA) were determined with an enzymatic assay (INstruchemie, Delfzijl, The Netherlands). HbA1c was determined in whole blood by hospital Gelderse Vallei (Ede, The Netherlands).

2.7. Habitual Dietary Intake and Sweet-Taste Preference

Habitual dietary intake including alcohol consumption was assessed by a validated 131-item semi-quantitative food frequency questionnaire (FFQ) [37,38]. Preference for sweet foods was assessed by a digital food preference ranking task [39]. Sweet-taste preference scores range from 1.5 to 3.5, with higher scores indicating greater preference for sweet foods compared to savory foods.

2.8. Statistical Analyses

Normality of variables was visually inspected using residual Q-Q plots. Skewed variables (plasma FGF21, VAT/SAT ratio, IHL, iAUC plasma glucose, fasting plasma insulin, iAUC plasma insulin, fasting plasma triglycerides, fasting plasma FFA, HOMA-IR, ASAT, and γGT) were log transformed (log2) to improve normality. Treatment effects on fasting FGF21 levels were analyzed using a general linear model for univariate analysis (ANCOVA), with baseline FGF21 level as covariate. Treatment effects on the postprandial FGF21 response were analyzed using ANOVA with the change in postprandial response as dependent variable. Correlations between variables were tested using Spearman's correlation coefficient. Differences in plasma FGF21 levels between tertiles of nutrient intake were analyzed using ANCOVA with adjustment for gender, and LSD post-hoc testing was used if overall differences were statistically significant. To evaluate the robustness of the results, we performed sensitivity analyses excluding outliers. Outliers were defined as plasma FGF21 levels that deviated more than two standard deviations (SD) from the mean. Data analysis was performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, US). Two-tailed $p < 0.05$ was considered statistically significant.

3. Results

A total of 100 subjects completed the study (n = 6 drop-outs in HQ, n = 1 drop-out in LQ, and n = 3 drop-outs in the control group [32]). Plasma FGF21 data were available from 98 subjects. Approximately half of the study population were women, median age was 62 years and median BMI was 30.8 kg/m^2. Baseline demographics and clinical characteristics were similar across intervention groups (Table 1).

In five participants (n = 2 in HQ, n = 1 in LQ, and n = 2 in the control group), plasma FGF21 levels deviated more than 2 SD from the mean both in the fasting and postprandial state, and both at baseline and after 12 weeks of intervention. Another two participants (n = 1 in HQ and n = 1 in LQ) had deviating plasma FGF21 levels in the postprandial state at baseline or in the fasting state after 12 weeks of intervention, respectively.

Table 1. Baseline characteristics of study participants with available FGF21 data.

	High-Nutrient-Quality Diet (*n* = 34)	Low-Nutrient-Quality Diet (*n* = 38)	Control Group (*n* = 26)
Women, *n* (%)	18 (52.9%)	21 (55.3%)	13 (50.0%)
Age, years	62 (53,65)	64 (54, 65)	61 (56, 66)
BMI, kg/m^2	31.1 (28.7, 33.8)	30.8 (28.9, 33.4)	30.3 (28.1, 32.6)
Intra-hepatic lipid content, %	3.1 (1.4, 10.2)	4.9 (2.6, 9.7)	3.7 (2.1, 8.4)
NAFLD, *n* (%)	9 (26.5%)	14 (36.8%)	7 (26.9%)
HbA1c, mmol/mol	37 ± 3	36 ± 2	35 ± 3
HOMA-IR	2.7 (2.0, 4.9)	2.8 (1.6, 5.0)	2.6 (1.9, 4.4)
HOMA-IR > 2.5, *n* (%)	19 (55.9%)	21 (55.3%)	14 (53.8%)
Plasma glucose, mmol/L	5.7 ± 0.5	5.6 ± 0.7	5.7 ± 0.4
Plasma insulin, mU/L	10.9 (8.2, 17.5)	11.6 (7.0, 18.6)	10.9 (7.9, 17.0)
Plasma triglycerides, mmol/L	1.5 (1.1, 1.9)	1.7 (1.3, 2.3)	1.7 (1.4, 2.1)
Serum total cholesterol, mmol/L	5.5 ± 0.8	5.8 ± 1.0	5.5 ± 1.0
Serum HDL cholesterol, mmol/L	1.4 ± 0.4	1.3 ± 0.4	1.3 ± 0.4
Plasma free fatty acids, mmol/L	0.5 (0.4, 0.6)	0.4 (0.3, 0.5)	0.4 (0.3, 0.5)
Systolic blood pressure, mmHg	131 ± 15	126 ± 19	126 ± 14
Diastolic blood pressure, mmHg	76 ± 9	72 ± 8	75 ± 9
Alanine aminotransferase, U/L	24 (19, 33)	24 (20, 33)	25 (20, 33)
Aspartate aminotransferase, U/L	22 (19, 26)	22 (19, 25)	24 (20, 32)
Gamma-glutamyl transferase, U/L	23 (18, 33)	26 (18, 36)	23 (17, 32)

Data are presented as mean ± SD or median (25th percentile, 75th percentile). Abbreviations: BMI, body mass index; NAFLD, non-alcoholic fatty liver disease; HbA1c, glycated hemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; HDL, high-density lipoprotein.

The reported dietary intakes of participants on the ER diets were in agreement with the advised (macro)nutrient composition, with higher intakes of MUFA, polyunsaturated fat, plant-based protein, and fiber, and lower intakes of SFA and fructose in the high-nutrient-quality diet compared to the low-nutrient-quality diet [32].

3.1. Effects of Dietary Interventions on Plasma FGF21

As has been previously reported, 12 weeks of 25% ER resulted in substantial weight loss (mean ± SD: −8.4 ± 3.2 kg in HQ and −6.3 ± 3.9 kg in LQ) and concomitant improvements in markers of metabolic health, including more favorable abdominal fat

distribution, and reductions in IHL, fasting glucose, and fasting insulin levels [32]. On average, plasma FGF21 levels were not affected by the interventions, nor did the interventions affect the postprandial FGF21 response (Table 2; Figure 1). Pooling of the ER groups together yielded similar results (ER: Δ fasting FGF21 −0.11 ng/ml, 95% CI −0.20 to −0.01, p ER vs. control group = 0.29; Δ postprandial FGF21 response −0.02 ng/ml, 95% CI −0.10 to 0.06, p ER vs. control group = 0.62). Exclusion of outliers did not affect the results (all $p > 0.19$).

Table 2. Change in fasting plasma FGF21 and the postprandial FGF21 response upon a 12-week energy restriction diet (low or high nutrient quality) compared to a control group.

	Baseline [a]	Change after 12 Wks [b]	p-Value [c]
Fasting FGF21 (ng/mL)			
Control group (n = 26)	0.95 ± 1.86	−0.01 (−0.17, 0.16)	0.48
Low-Nutrient-Quality Diet (n = 38)	0.77 ± 1.89	−0.08 (−0.21, 0.05)	
High-Nutrient-Quality Diet (n = 34)	0.71 ± 1.92	−0.14 (−0.28, 0.004)	
Postprandial FGF21 (ng/mL)			
Control group (n = 26)	0.82 ± 2.00	0.00 (−0.15, 0.15)	0.57
Low-Nutrient-Quality Diet (n = 37)	0.71 ± 1.93	−0.09 (−0.21, 0.04)	
High-Nutrient-Quality Diet (n = 32)	0.64 ± 1.88	−0.10 (−0.24, 0.04)	
Postprandial FGF21 response (ng/mL)			
Control group (n = 26)	−0.12 ± 0.22	−0.02 (−0.15, 0.11)	0.86
Low-Nutrient-Quality Diet (n = 37)	−0.02 ± 0.27	0.01 (−0.10, 0.12)	
High-Nutrient-Quality Diet (n = 32)	−0.05 ± 0.34	0.03 (−0.09, 0.15)	

[a] Values are geometric means ± SD (fasting and postprandial FGF21) or means ± SD (postprandial FGF21 response).
[b] Values are (adjusted) means with 95% confidence intervals.
[c] Differences between the groups were analyzed using ANCOVA with baseline FGF21 levels as covariate (fasting and postprandial FGF21) or using univariate ANOVA (postprandial FGF21 response).

3.2. Postprandial Plasma FGF21 Response at Baseline

On average, plasma FGF21 tended to decrease 120 min after consumption of the mixed meal (Δ −0.06 ng/ml, 95% CI −0.11 to 0.001, p = 0.055), although inter-individual variation in response was high (Figure 2A). Exclusion of outliers strengthened this effect (Δ −0.12 ng/ml, 95% CI −0.23 to −0.02, p = 0.026).

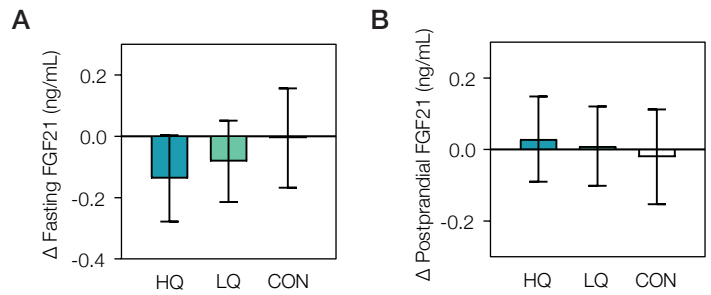

Figure 1. Effects of two 12-week energy restriction diets (HQ, high-nutrient-quality diet; LQ, low-nutrient-quality diet) or control group (CON) on fasting and postprandial plasma FGF21 levels. (A) Geometric mean (95% CI) change in fasting plasma FGF21 upon a 12-week diet, as tested by ANCOVA with adjustment for baseline fasting FGF21 levels. (B) Geometric mean (95% CI) change in postprandial FGF21 response (Δ120–0 min) to a mixed meal upon a 12-week diet, as tested by univariate ANOVA.

3.3. Plasma FGF21 and Correlations with Markers of Metabolic Health

At baseline, fasting plasma FGF21 concentrations were inversely correlated to plasma FFA (ρ = −0.22, p = 0.03), and not correlated to BMI, IHL, HOMA-IR or any of the other assessed cardiometabolic parameters (Figure 2C and Figure S1; Table S1). The change in plasma FGF21 from the fasting state to 120 min postprandially was inversely correlated to fasting plasma glucose, fasting insulin, HOMA-IR, VAT and ASAT (ρ = −0.34 to −0.23, all $p < 0.05$), but did not correlate with IHL or the postprandial response of plasma glucose or insulin (Figure 2C and Figure S1; Table S1). Exclusion of outliers attenuated the inverse correlations of the postprandial FGF21 response with plasma glucose (ρ = −0.19, p = 0.069) and ASAT (ρ = −0.20, p = 0.051), annulled the correlation with VAT (ρ = −0.18, p = 0.14), and did not affect the other correlations.

To further explore the relationship between the postprandial FGF21 response and HOMA-IR, we stratified the total study population into insulin-sensitive (n = 44) and insulin-resistant (n = 53) subjects based on HOMA-IR ≤ 2.5 and >2.5 [38]. In insulin sensitive subjects, FGF21 did not change from fasting to 120 min postprandially, while in insulin resistant subjects, FGF21 declined 120 min after consumption of the mixed meal, also after adjustment for fasting FGF21, age, gender, and BMI (Δ −0.03 ng/ml, 95% CI −0.06 to 0.11 vs. Δ −0.13 ng/ml, 95% CI −0.20 to −0.05; p = 0.012) (Figure 2B). Exclusion of outliers (p = 0.007) or additional adjustment for NAFLD status (IHL ≤ 5.56% or > 5.56% [39]) or IHL did not affect this result (p = 0.013 and p = 0.018, respectively).

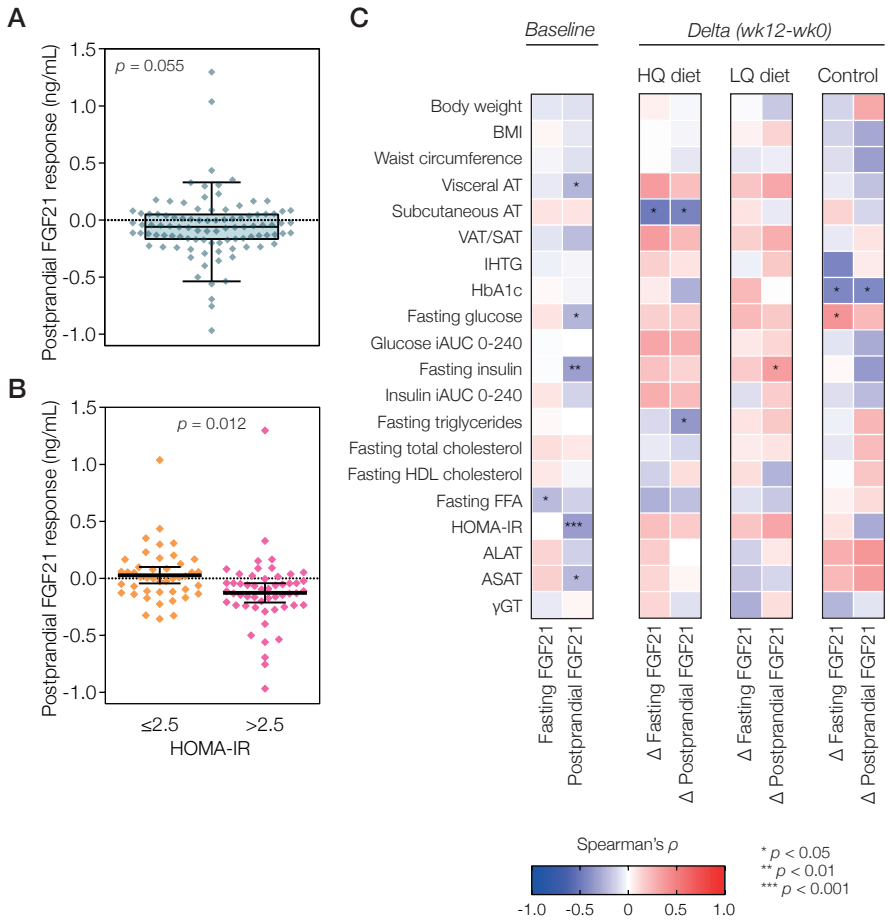

Figure 2. Postprandial FGF21 response at baseline and correlations between plasma FGF21 and markers of metabolic health. (A) Box plot with individual data points of the change in plasma FGF21 levels from the fasting state to 120 min after the consumption of the mixed meal in the complete population at baseline (p = 0.055, as tested using a paired-samples T-test). The box plot represents the 5th percentile, first quartile, median, third quartile, and 95th percentile. (B) Individual postprandial FGF21 responses according to baseline HOMA-IR: ≤2.5 or >2.5 (p = 0.012 for the difference between groups, as tested with ANCOVA, adjusted for fasting FGF21, age, gender, and BMI. (C) Spearman correlations between fasting FGF21 and the postprandial FGF21 response with cardiometabolic parameters at baseline (left) and in response to a 12-week intervention (right). Abbreviations: BMI, body mass index; AT, adipose tissue; IHL, intra-hepatic lipid content; HbA1c, glycated hemoglobin A1c; iAUC, incremental area under the curve; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; ALAT, aminotransferase; ASAT, aspartate aminotransferase; γGT, gamma-glutamyl transferase.

Next to testing correlations at baseline, we also explored correlations between the change in fasting and postprandial FGF21 response and change in markers of metabolic health. In the high-nutrient-quality diet group, change in both fasting FGF21 and the postprandial FGF21 response upon the 12 weeks of intervention was inversely correlated to the reduction in abdominal SAT (ρ = −0.49, p = 0.012; ρ = −0.45, p = 0.023, respectively) (Figure 2C; Table S2; Table S3). Furthermore, in the high-nutrient-quality diet group, the change in postprandial FGF21 response was inversely correlated to the reduction in fasting plasma triglycerides (ρ = −0.36, p = 0.041) (Figure 2C; Table S3). In the low-nutrient-quality diet, the change in postprandial FGF21 response was positively correlated to the reduction in fasting insulin levels (ρ = 0.35, p = 0.034) (Figure 2C; Table S3). In the control group, changes in both fasting and postprandial FGF21 response were inversely correlated to change in HbA1c, and change in fasting FGF21 levels was positively correlated to change in fasting glucose levels (Figure 2C; Table S3). These latter three correlations, however, were driven by data points from two participants, and exclusion of these data points resulted in a loss of significant correlations (ρ = −0.30 to 0.25, p > 0.13).

Exclusion of outliers resulted in positive correlations between the change in fasting FGF21 and the change in fasting insulin, iAUC insulin, and HOMA-IR in the high-nutrient-quality diet group (ρ = 0.36 to 0.51, all p < 0.05). In addition, exclusion of outliers attenuated the inverse correlation between the change in postprandial FGF21 response and change in fasting plasma triglycerides in the high-nutrient-quality diet group (ρ = −0.31, p = 0.097), annulled the positive correlation between change in postprandial FGF21 response and change in fasting insulin in the low-nutrient-quality diet group (ρ = 0.27, p = 0.11), and did not affect the other correlations.

3.4. Plasma FGF21 and Habitual (Macro)Nutrient Intake and Sweet-Taste Preference

Since FGF21 has been linked to (macro)nutrient intake and preference, we compared fasting plasma FGF21 levels between tertiles of habitual nutrient intake, alcohol consumption, and sweet-taste preference. Fasting FGF21 levels were lower in individuals in the highest tertile of habitual polysaccharide intake compared to individuals in lower tertiles of polysaccharide intake (overall p = 0.022; tertile 3 vs. tertile 1, p = 0.035; tertile 3 vs. tertile 2, p = 0.009; Figure 3C). FGF21 levels did not differ between individuals in tertiles of habitual intake of carbohydrates, protein, fat, mono- and disaccharides (sugars), or alcohol consumption (Figure 3). In addition, plasma FGF21 levels did not differ according to sweet-taste preference (Figure 3G). The ranges and means of the tertiles of habitual nutrient intake, alcohol consumption, and sweet-taste preference are reported in Table S4. Exclusion of outliers attenuated the differences in fasting FGF21, according to habitual polysaccharide intake (overall p = 0.098; tertile 3 vs. tertile 1, p = 0.066; tertile 3 vs. tertile 2, p = 0.063).

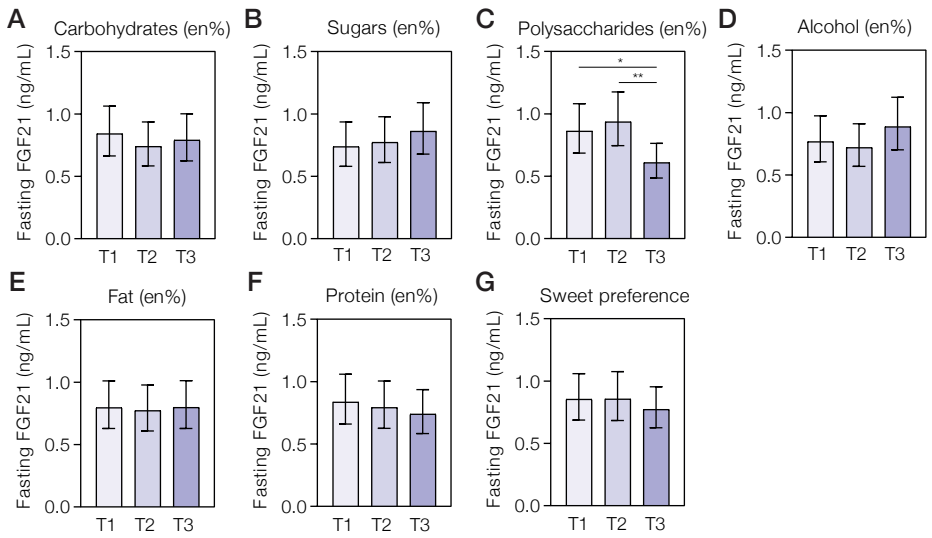

Figure 3. Fasting plasma FGF21 levels (adjusted geometric means with 95% CI) according to tertiles (T1: lowest tertile, T3: highest tertile) of habitual (macro)nutrient intake as % of daily energy intake (en%) (A–C,E,F), habitual alcohol consumption (D), and sweet-taste preference (G). FGF21 levels were lower in the highest tertile of polysaccharide intake compared to the lower tertiles (overall p = 0.022; tested by ANCOVA with adjustment for gender and LSD post-hoc testing; * p = 0.035; ** p = 0.009) and did not differ between tertiles of other nutrient intakes.

3.5. Baseline Plasma FGF21 and Weight Loss

In an explorative analysis, we examined whether fasting plasma FGF21 and the FGF21 postprandial response at baseline were associated with weight loss after 12 weeks of ER intervention. Fasting plasma FGF21 levels at baseline were not correlated to weight loss in either of the ER groups (HQ: ρ = −0.01, p = 0.96; LQ: ρ = −0.27, p = 0.10; HQ and LQ combined: ρ = −0.08, p = 0.50 Figure 4A). The postprandial change in FGF21 at baseline was borderline positively associated with weight change in the low-nutrient-quality diet group (ρ = 0.30, p = 0.065) but not in the high-nutrient-quality diet group or in the two groups combined (HQ: ρ = −0.23, p = 0.19; HQ and LQ combined: ρ = 0.06, p = 0.63 Figure 4B). Exclusion of outliers resulted in an inverse correlation between fasting FGF21 at baseline and weight loss (ρ = −0.33, p = 0.048) and attenuated the correlation with the postprandial FGF21 response in the low-nutrient-quality diet group (ρ = 0.27, p = 0.10).

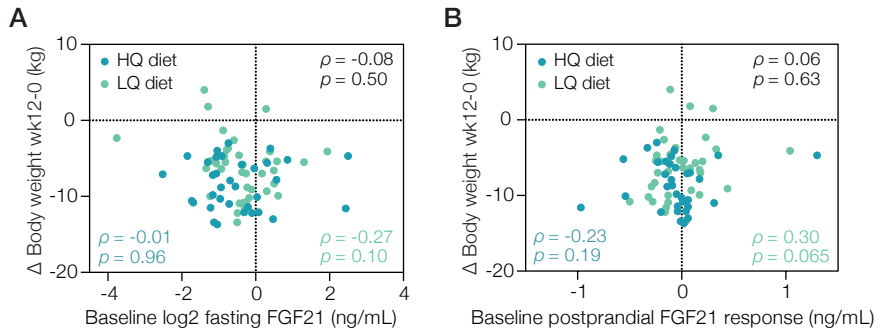

Figure 4. Scatter plots of fasting plasma FGF21 levels (A) and the postprandial FGF21 response (B) at baseline with weight change after 12 weeks of intervention on the high-nutrient-quality diet (HQ, dark green) and low-nutrient-quality diet (LQ, light green). Correlations were tested using Spearman correlation coefficients (LQ and HQ group combined in black).

4. Discussion

In this study, we investigated the effects of two 12-week energy restriction (ER) diets differing in nutrient quality on fasting and postprandial plasma FGF21 levels, and explored correlations between plasma FGF21 and markers of metabolic health. Neither overall ER nor high vs. low nutrient quality of the ER diets affected circulating fasting or postprandial FGF21 levels. Diet-induced weight loss and liver fat reduction were not accompanied by changes in fasting or postprandial plasma FGF21. Fasting plasma FGF21 was not correlated to markers of metabolic health at baseline, but the postprandial FGF21 response to the mixed meal was inversely correlated to fasting glucose, insulin, HOMA-IR, visceral AT and the liver enzyme ASAT. In addition, we assessed associations between habitual dietary intake and circulating FGF21 levels. Fasting FGF21 levels were lowest in individuals with the highest intake of polysaccharides, but did not differ according to intake of mono- and disaccharides, alcohol consumption, or sweet-taste preference.

Despite substantial weight loss of 7.3 kg on average and concomitant health improvements [32], 12 weeks of 25% energy reduction did not affect fasting FGF21 levels. Previous weight loss trials have reported conflicting findings, with some trials reporting a reduction in fasting FGF21 levels upon diet-induced weight loss [9,10,11,12,13], and others reporting an increase [40] or no effect on circulating FGF21 levels [14,15,16,17,18,19,20]. Generally, the studies that report a change in plasma fasting FGF21 were performed in individuals with more severely impaired metabolic health (e.g., T2DM, NAFLD, morbid obesity), who typically have elevated FGF21 levels

and thus more room for improvement compared to individuals in this study. In addition, the degree of weight loss, as well as the content and composition of the intervention diets may contribute to disagreement between studies. Weight loss as a result of bariatric surgery, which is commonly larger than weight loss achieved by ER, such as in our study, is also not consistently accompanied by changes in FGF21 levels [41]. It thus seems that weight loss and accompanying health improvements do not consistently affect fasting FGF21 levels.

Although fasting FGF21 levels were not correlated to liver fat or markers of glucose or lipid metabolism at baseline, change in fasting plasma FGF21 levels upon the 12-week intervention was inversely correlated to change in SAT, with larger reductions in SAT being accompanied by an increase in fasting plasma FGF21. In contrast, after the exclusion of five outliers, the reductions in HOMA-IR and both fasting and postprandial plasma insulin upon the intervention were correlated to a decrease in fasting FGF21, indicating that change in fasting FGF21 may be a marker for improvement in insulin sensitivity. These correlations, however, were present only in the high-nutrient-quality diet group, and it is unclear why we did not find similar correlations in the low-nutrient-quality diet group, given that the reductions in SAT, plasma insulin, and HOMA-IR were comparable upon the two ER diets. In addition, excluding participants with deviating values from analysis may lead to bias and therefore, these results should be interpreted with caution.

At baseline, the postprandial FGF21 response was inversely correlated to insulin resistance as estimated by HOMA-IR, with more insulin resistant individuals exhibiting a larger postprandial decline in plasma FGF21 compared to insulin sensitive individuals. Circulating FGF21 levels have previously been reported to modestly decrease for 1 to 4 h after ingestion of meals containing fat, protein, or a combination of macronutrients [13,42,43,44]. In line with our findings, individuals with T2DM have been reported to have a more pronounced decline in postprandial FGF21 levels after a mixed meal compared to individuals with normal glucose metabolism [13]. These observations suggest that circulating FGF21 concentrations in response to mixed meals may be less well-controlled in impaired metabolic health.

The regulation of fasting and postprandial FGF21 levels in response to different nutrients and nutrient combinations is complex and not fully understood [1,2]. Overall, plasma FGF21 levels appear to display a circadian rhythm, with peak levels during fasting and lower levels during feeding [45]. During fasting, liver-derived FGF21 is primarily regulated by the transcription factor PPARα, and intake of fat, protein or a mixed meal results in diminished PPARα-mediated FGF21 secretion, possibly (partly) via a reduction in plasma FFA levels [15,45,46]. High intake of pure simple sugars or alcohol forms an exception; these nutrients acutely elevate plasma FGF21 [30,47,48,49], likely via activation of hepatic ChREBP [29,50]. Similar to our findings of a more pronounced postprandial response to a mixed meal in IR, plasma FGF21

excursions after pure fructose or glucose ingestion have been found to be larger in individuals with metabolic syndrome compared to healthy individuals [47]. In addition, the postprandial FGF21 response to fructose has been positively correlated to measures of hepatic and adipose tissue IR [48]. Circulating FGF21 in response to acute nutritional challenges may thus be a marker of metabolic health. Various factors including insulin, glucagon, adiponectin, and FFA appear to be involved in the regulation of FGF21 [1]. Greater FGF21 excursions in response to acute (nutritional) challenges, i.e., poor control of plasma FGF21 levels, in conditions of impaired metabolic health may reflect the disturbed control of the signals that regulate FGF21. Further research into the mechanisms that underlie altered FGF21 dynamics in impaired metabolic health is warranted.

If the postprandial FGF21 response is a marker for metabolic health, it could be expected that the substantial weight loss and concomitant health improvements after 12 weeks of ER in this study would be accompanied by a reduction in the postprandial FGF21 response. We, however, found no effects of the 12-week ER interventions on postprandial FGF21. A trial in individuals with T2DM and morbid obesity did report that weight loss of ~6.5 kg upon a 3-week very-low-calorie diet was accompanied by a less pronounced decline in postprandial in FGF21 levels up to 3 h after a mixed meal [13]. Our null findings may be due to our relatively less metabolically impaired study population and therefore less pronounced health improvements compared to the study in T2DM patients. In addition, we observed great inter-individual variation in the postprandial FGF21 response, with on average a modest decrease in plasma FGF21 levels 120 min after the mixed meal. Still, in approximately one third of the subjects, plasma FGF21 did not change or increased postprandially, which highlights the complexity of plasma FGF21 regulation and the challenge of interpreting plasma FGF21 (dynamics).

Recently, FGF21 levels have been suggested to be predictive of weight loss. We, however, did not find baseline fasting nor postprandial FGF21 concentrations to correlate with weight loss upon 12 weeks of ER. After exclusion of five outliers, we did observe that higher baseline fasting FGF21 was correlated to larger weight loss in the low- and not the high-nutrient-quality diet group, although these results should be interpreted with caution. In the DIETFITS trial, a trial in which 609 overweight or obese adults were randomized to follow a low-fat or low-carbohydrate weight-loss diet for 3 months, higher baseline FGF21 levels were associated with larger weight loss [21]. The inconsistent findings in our study might be due to limited power. In addition, in the DIETFITS trial, participants were instructed to drastically reduce their carbohydrate or fat intake, while there were no specific instructions regarding caloric intake. As FGF21 has been suggested to regulate energy homeostasis in humans by amongst others decreasing caloric intake [1], the association between baseline FGF21 and weight loss may thus partly result from differences in FGF21-mediated

caloric intake. In our study, participants were explicitly instructed and monitored to reduce caloric intake by 25%, which leaves less room for regulation of caloric intake by FGF21.

FGF21 has also been implicated in the regulation of macronutrient intake. We found that individuals with higher habitual intake of polysaccharides had lower fasting FGF21 levels, but we found no associations between FGF21 and sweet-taste preference or habitual intake of mono- and disaccharides. Previously, higher soda consumption has been associated with lower circulating FGF21 [28], and FGF21 gene variants have been related to higher total carbohydrate intake [24,25,26,27]. The only GWAS study that made a distinction between type of carbohydrates [26], however, found that the association between FGF21 gene variants and carbohydrate intake could be attributed solely to higher intake of mono- and disaccharides and not to higher intake of polysaccharides. Animal studies also indicate that FGF21 regulates intake of mono- and disaccharides specifically [29], so our finding of lower plasma FGF21 levels in individuals with high intake of polysaccharides remains unexplained.

Strengths of this study include its RCT design, with—next to two ER dietary intervention groups—a control group that did not receive dietary advice, which reduces bias and confounding factors. In addition, this study had relatively few drop-outs and compliance to ER was high, as is demonstrated by the substantial weight loss that participants achieved.

This was a secondary analysis of an RCT designed to study effects of ER diets differing in nutrient quality on metabolic health outcomes, and the original sample size calculation was based on the power to detect differences in IHL [32]. Given the large inter-individual variation in plasma FGF21 levels, this study may have been underpowered to detect effects on plasma FGF21. Further large trials are thus needed to confirm our findings. Another limitation may be that we measured total plasma FGF21 protein, rather than bioactive FGF21. In the circulation, FGF21 is rapidly degraded by enzymatic cleavage, rendering it inactive [51]. Measuring bioactive rather than total FGF21 concentrations may be more physiologically relevant. Furthermore, we only measured postprandial FGF21 levels at a single timepoint (2 h after consumption of the mixed meal), and do not know the plasma FGF21 levels in the time between. We, however, expect that plasma FGF21 steadily decreased throughout the 2 h after consumption, since a previous study with more frequent sampling times showed a continuous decline in plasma FGF21 up to 3 h after a mixed meal [13]. Lastly, we did not assess physical activity level and therefore cannot rule out that differences in physical activity level at baseline or follow-up may have affected plasma FGF21 levels, given that exercise modifies circulating FGF21 levels [52]. However, we consider it unlikely that the ER groups differed in physical activity level at baseline or follow-up, because (1) interventions were randomly allocated, (2) participants were unaware of the high-nutrient-quality vs. low-nutrient-quality distinction, and (3) participants were instructed to maintain their habitual physical activity level throughout the study.

5. Conclusions

In conclusion, weight loss and concomitant health improvements upon a 12-week 25% ER diet were not accompanied by changes in fasting or postprandial plasma FGF21 levels in middle-aged individuals with abdominal obesity. Neither overall ER nor nutrient quality affected plasma FGF21. In addition, we found no robust evidence that fasting plasma FGF21 is a marker for metabolic health status. We did find indications that circulating FGF21 dynamics in response to a nutritional challenge may reflect metabolic health status. FGF21′s metabolic regulation and functions are greatly complex and further research on the potential of circulating FGF21 dynamics as a marker of metabolic health is warranted.

Author Contributions

Conceptualization and methodology, A.G., S.S., D.E., C.C.J.R.M., E.S., M.M. (Monica Mars), M.M. (Marco Mensink) and L.A.A.; investigation, S.S., D.E. and E.S.; formal analysis, visualization and writing—original draft preparation, A.G.; supervision and writing—review and editing, L.A.A. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Division of Human Nutrition and Health, Wageningen University, The Netherlands and received no external funding.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of Wageningen University (protocol code NL44614.081.13, approved on 3 June 2014). The study is registered at ClinicalTrials.gov as NCT02194504.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Data presented in this manuscript are available upon reasonable request.

Acknowledgments

We thank the volunteers that participated in this study and the Wageningen Belly Fat Study team. We thank Nhien Ly and Lisette van de Lende for technical assistance with the biochemical analyses and Suzanne de Bruijn and Fildzah Karunia Putri for assistance with the food preference ranking test.

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary materials

Table S1. Correlations between both fasting plasma FGF21 and the postprandial response of plasma FGF21 and markers of metabolic health

	Fasting plasma FGF21			Postprandial plasma FGF21 response[b]		
	Spearman's ρ	p	n	Spearman's ρ	p	n
Body weight	-0.07	0.469	98	-0.09	0.376	97
BMI	0.03	0.786	98	-0.07	0.491	97
Waist circumference	-0.03	0.789	98	-0.09	0.397	97
Visceral AT	-0.06	0.638	69	**-0.24**	**0.046**	69
Subcutaneous AT	0.09	0.447	69	0.09	0.443	69
VAT/SAT ratio[a]	-0.08	0.491	69	-0.21	0.081	69
IHL[a]	-0.04	0.734	79	-0.03	0.781	79
HbA1c	0.02	0.810	98	-0.03	0.773	97
Fasting glucose	0.09	0.367	98	**-0.24**	**0.020**	97
iAUC glucose[a]	-0.01	0.953	94	-0.00	0.966	93
Fasting insulin[a]	-0.01	0.905	98	**-0.33**	**0.001**	97
iAUC insulin[a]	0.08	0.457	98	-0.13	0.204	97
Fasting triglycerides[a]	0.02	0.830	98	-0.001	0.992	97
Fasting total cholesterol	0.10	0.315	98	0.07	0.487	97
Fasting HDL cholesterol	0.07	0.486	98	-0.03	0.766	97
Fasting FFAs[a]	**-0.22**	**0.031**	96	-0.14	0.182	95
HOMA-IR[a]	0.00	0.985	98	**-0.34**	**0.001**	97
ALAT	0.14	0.172	98	-0.14	0.169	97
ASAT[a]	0.15	0.151	98	**-0.23**	**0.021**	97
γGT[a]	-0.06	0.578	98	0.03	0.756	97

[a] Log2 transformed to improve normality
[b] Calculated as the change from plasma FGF21 in the fasted state to 120 minutes after the high-fat mixed meal
Abbreviations: BMI, body mass index; AT, adipose tissue; IHL, intra-hepatic lipid content; HbA1c, glycated hemoglobin A1c; iAUC, incremental area under the curve; HDL, high-density lipoprotein; FFAs, free fatty acids; HOMA-IR, homeostatic model assessment of insulin resistance; ALAT, aminotransferase; ASAT, aspartate aminotransferase; γGT, gamma-glutamyl transferase.

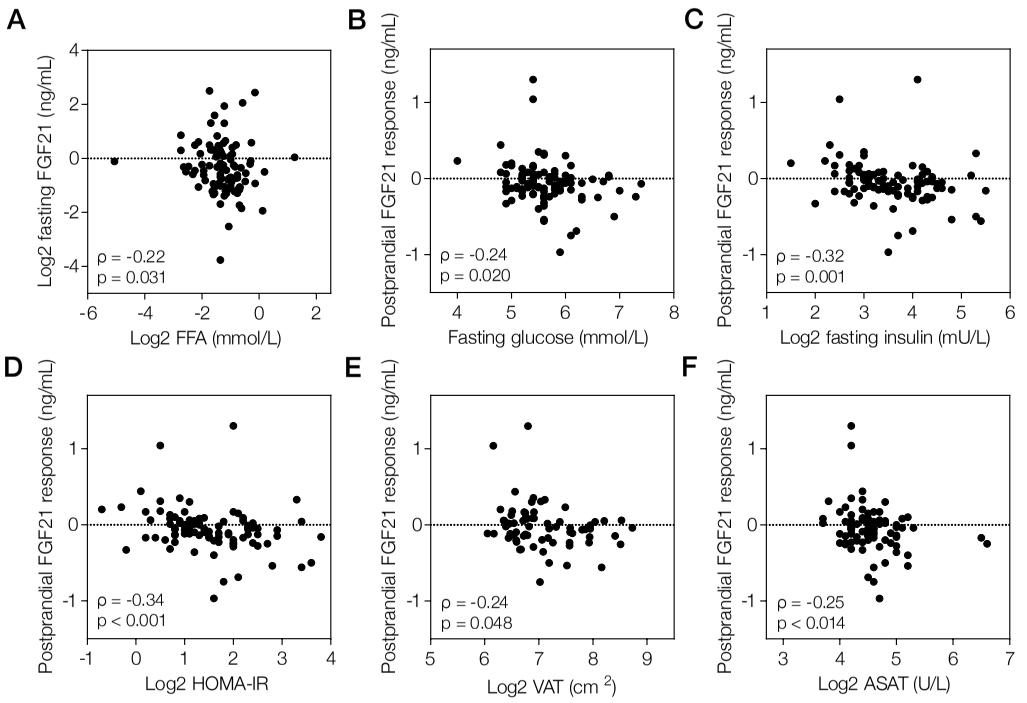

Figure S1. Scatter plots of fasting plasma FGF21 and the postprandial plasma FGF21 response upon a liquid high-fat mixed meal with markers of metabolic health. Plasma free fatty acids (FFA) were inversely correlated to fasting plasma FGF21 (A). Fasting plasma glucose (B), fasting plasma insulin (C), homeostatic model assessment of insulin resistance (HOMA-IR) (D), visceral adipose tissue (VAT) (E), and serum aspartate aminotransferase (ASAT) (F) were inversely correlated to the postprandial plasma FGF21 response. The postprandial FGF21 response was calculated as the change from plasma FGF21 in the fasted state to 120 minutes after the high-fat mixed meal. ρ denotes the Spearman correlation coefficient.

Table S2. Correlations between the change in fasting plasma FGF21 and the change in metabolic health markers upon a 12-week diet

	Δ Fasting plasma FGF21								
	Control group			Low-nutrient-quality diet			High-nutrient-quality diet		
Δ	Spearman's ρ	*p*	*n*	Spearman's ρ	*p*	*n*	Spearman's ρ	*p*	*n*
Body weight	-0.13	0.535	26	-0.01	0.931	38	0.05	0.799	34
BMI	-0.13	0.540	26	0.04	0.833	38	0.01	0.968	34
Waist circumference	-0.10	0.642	26	-0.07	0.670	37	0.01	0.964	33
VAT	-0.06	0.837	16	0.20	0.311	27	0.36	0.074	26
SAT	0.14	0.602	16	0.09	0.650	27	**-0.49**	**0.012**	26
VAT/SAT ratio	-0.06	0.820	16	0.15	0.458	27	0.36	0.072	26
IHL	-0.44	0.058	19	-0.04	0.829	33	0.15	0.428	29
HbA1c	**-0.43**	**0.029**	26	0.24	0.143	38	0.06	0.741	34
Fasting glucose	**0.40**	**0.044**	26	0.24	0.147	38	0.15	0.403	34
iAUC glucose	-0.08	0.696	26	0.07	0.694	38	0.32	0.063	34
Fasting insulin	0.02	0.919	26	0.17	0.320	38	0.21	0.250	33
iAUC insulin	-0.10	0.643	26	-0.10	0.568	38	0.29	0.096	34
Fasting triglycerides	-0.04	0.860	26	0.09	0.586	38	-0.11	0.530	34
Fasting total cholesterol	-0.07	0.751	26	0.06	0.704	38	-0.06	0.748	34
Fasting HDL cholesterol	-0.01	0.948	26	0.10	0.560	38	-0.14	0.438	34
Fasting FFAs	0.04	0.850	26	-0.09	0.580	38	-0.26	0.157	31
HOMA-IR	0.09	0.675	26	0.20	0.231	38	0.22	0.227	33
ALAT	0.28	0.170	26	-0.14	0.416	38	0.16	0.371	34
ASAT	0.24	0.229	26	-0.18	0.271	38	0.13	0.461	34
γGT	-0.24	0.232	26	-0.27	0.101	38	0.13	0.460	34

Abbreviations: BMI, body mass index; AT, adipose tissue; IHL, intra-hepatic lipid content; HbA1c, glycated hemoglobin A1c; iAUC, incremental area under the curve; HDL, high-density lipoprotein; FFAs, free fatty acids; HOMA-IR, homeostatic model assessment of insulin resistance; ALAT, aminotransferase; ASAT, aspartate aminotransferase; γGT, gamma-glutamyl transferase.

Table S3. Correlations between change in the postprandial plasma FGF21 response upon a liquid high-fat mixed meal and the change in metabolic health markers upon a 12-week diet

	Δ Postprandial plasma FGF21 response[a]								
	Control group			Low-nutrient-quality diet			High-nutrient-quality diet		
Δ	Spearman's ρ	*p*	*n*	Spearman's ρ	*p*	*n*	Spearman's ρ	*p*	*n*
Body weight	0.30	0.131	26	-0.17	0.316	37	-0.02	0.905	32
BMI	-0.29	0.151	26	0.14	0.399	37	-0.03	0.879	32
Waist circumference	-0.33	0.098	26	-0.05	0.752	36	-0.07	0.697	31
VAT	-0.19	0.478	16	0.31	0.124	26	0.22	0.294	25
SAT	-0.12	0.656	16	-0.06	0.774	26	-0.45	0.023	25
VAT/SAT ratio	0.09	0.753	16	0.28	0.165	26	0.24	0.250	25
IHL	0.06	0.814	19	0.18	0.323	32	0.09	0.665	28
HbA1c	**-0.42**	**0.031**	26	0.01	0.958	37	-0.26	0.154	32
Fasting glucose	0.24	0.239	26	0.18	0.288	37	0.16	0.388	32
iAUC glucose	-0.28	0.162	26	0.13	0.460	37	0.29	0.111	32
Fasting insulin	-0.35	0.077	26	**0.35**	**0.034**	37	0.14	0.454	31
iAUC insulin	-0.21	0.296	26	0.16	0.347	37	0.23	0.201	32
Fasting triglycerides	0.25	0.226	26	0.18	0.280	37	**-0.36**	**0.041**	32
Fasting total cholesterol	0.23	0.260	26	0.09	0.576	37	-0.12	0.504	32
Fasting HDL cholesterol	0.19	0.340	26	-0.25	0.131	37	0.10	0.596	32
Fasting FFAs	0.11	0.583	26	-0.17	0.304	37	-0.20	0.309	29
HOMA-IR	-0.28	0.166	26	0.32	0.054	37	0.17	0.351	31
ALAT	0.39	0.050	26	0.07	0.699	37	0.00	0.998	32
ASAT	0.35	0.084	26	-0.12	0.476	37	0.03	0.871	32
γGT	-0.08	0.694	26	0.10	0.555	37	-0.09	0.622	32

[a] Calculated as baseline vs. post-intervention difference in the change from plasma FGF21 in the fasted state to 120 minutes after the high-fat mixed meal
Abbreviations: BMI, body mass index; AT, adipose tissue; IHL, intra-hepatic lipid content; HbA1c, glycated hemoglobin A1c; iAUC, incremental area under the curve; HDL, high-density lipoprotein; FFAs, free fatty acids; HOMA-IR, homeostatic model assessment of insulin resistance; ALAT, aminotransferase; ASAT, aspartate aminotransferase; γGT, gamma-glutamyl transferase

Table S4. Ranges and means of tertiles of habitual nutrient intake, alcohol consumption, and sweet-taste preference

	Tertile 1			Tertile 2			Tertile 3		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Fat, en%	26.4 – 36.8	33.5	2.7	36.9 – 40.4	38.6	1.2	40.8 – 54.5	44.8	3.8
Protein, en%	11.7 – 15.0	13.8	0.8	15.0 – 16.6	15.8	0.5	16.7 – 27.9	18.7	2.3
Carbohydrates, en%	23.5 – 37.2	32.9	3.4	37.5 – 41.3	39.5	1.1	41.4 – 50.3	45.2	2.9
Mono- and disaccharides, en%	4.6 – 16.1	13.4	2.5	16.3 – 19.5	18.0	0.9	19.6 – 30.0	23.1	2.7
Polysaccharides, en%	11.7 – 19.8	16.7	2.1	20.0 – 22.6	21.3	0.8	22.7 – 31.0	25.2	2.0
Alcohol, en%	0.0 – 1.1	0.3	0.4	1.1 – 4.7	2.7	1.1	4.9 – 14.6	6.8	2.3
Sweet-taste preference, AU	2.0 – 2.6	2.4	0.2	2.6 – 2.8	2.7	0.1	2.8 – 3.3	3.0	0.1

Abbreviations: SD, standard deviation; en%, % of daily energy intake

3

Effects of a 12-week whole-grain or refined wheat intervention on plasma acylcarnitines, bile acids and signaling lipids, and association with liver fat: A post-hoc metabolomics study of a randomized controlled trial

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Frontiers in Nutrition **2022**, Oct 13;*9*:1026213

Abstract

Background: We previously showed that whole-grain wheat (WGW) consumption had beneficial effects on liver fat accumulation, as compared to refined wheat (RW). The mechanisms underlying these effects remain unclear.

Objective: In this study, we investigated the effects of WGW vs. RW consumption on plasma metabolite levels to explore potential underlying mechanisms of the preventive effect of WGW consumption on liver fat accumulation.

Methods: Targeted metabolomics of plasma obtained from a concluded 12-week double-blind, randomized controlled trial was performed. Fifty overweight or obese men and women aged 45–70 years with mildly elevated levels of plasma cholesterol were randomized to either 98 g/d of WGW or RW products. Before and after the intervention, a total of 89 fasting plasma metabolite concentrations including acylcarnitines, trimethylamine-N-oxide (TMAO), choline, betaine, bile acids, and signaling lipids were quantified by UPLC-MS/MS. Intrahepatic triglycerides (IHTG) were quantified by ^{1}H-MRS, and multiple liver markers, including circulating levels of β-hydroxybutyrate, alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyltransferase (γ-GT), serum amyloid A (SAA), and C-reactive protein, were assessed.

Results: The WGW intervention increased plasma concentrations of four out of 52 signaling lipids—lysophosphatidic acid C18:2, lysophosphatidylethanolamine C18:1 and C18:2, and platelet-activating factor C18:2—and decreased concentrations of the signaling lipid lysophosphatidylglycerol C20:3 as compared to RW intervention, although these results were no longer statistically significant after false discovery rate (FDR) correction. Plasma concentrations of the other metabolites that we quantified were not affected by WGW or RW intervention. Changes in the above-mentioned metabolites were not correlated to change in IHTG upon the intervention.

Conclusion: Plasma acylcarnitines, bile acids, and signaling lipids were not robustly affected by the WGW or RW interventions, which makes them less likely candidates to be directly involved in the mechanisms that underlie the protective effect of WGW consumption or detrimental effect of RW consumption on liver fat accumulation. Clinical trial registration: [www.ClinicalTrials.gov], identifier [NCT02385149].

Introduction

In concurrence with the global obesity epidemic, prevalence rates of non-alcoholic fatty liver (NAFL) are on the rise (1). A 2016 study estimated that one in four adults has NAFL worldwide (1). NAFL is defined as excessive hepatic fat accumulation not caused by significant alcohol consumption or other diseases or medication known to induce steatosis (2). NAFL can progress to steatohepatitis (NASH), which is one of the leading causes of chronic liver disease and increases morbidity and mortality from cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) (2–6).

Hepatic fat accumulates when hepatic lipid storage exceeds lipid disposal. Sources of lipid influx include dietary fatty acids from intestinally derived chylomicron remnants, circulating free fatty acids (FFA) derived from adipose tissue lipolysis, and newly synthesized lipids from carbohydrates or amino acids by hepatic de novo lipogenesis (7). Lipids are cleared from the liver via either mitochondrial β-oxidation or export into the circulation in very low-density lipoprotein (VLDL) particles (7). Key modifiable risk factors for a disequilibrium between hepatic lipid storage and clearance resulting in liver fat accumulation include abdominal obesity, insulin resistance, and dyslipidemia, although the direction of causality between hepatic steatosis and other metabolic abnormalities is unclear and may best be described as bidirectional (2, 6, 8, 9).

The prevention and treatment of hepatic steatosis is primarily based on weight loss (2), although dietary modification has also been shown to affect liver fat independently of weight change (10). A modification that has been suggested to benefit liver health is replacing refined grains with whole grains (11, 12). Compared to refined grains, whole grains contain higher amounts of various nutrients and phytochemicals that may benefit liver health, such as fibers, betaine, and choline (11). Both betaine and choline are directly involved in hepatic lipid metabolism. Choline's major fate is incorporation into phosphatidylcholine (PC), which is required for packaging and export of lipids from the liver in VLDL, and thereby is essential for hepatic lipid disposal (13). Choline can also be oxidized to betaine. Betaine in turn acts as a methyl-group donor in the methionine-homocysteine cycle in the liver, which plays a central role in de novo synthesis of PC (14, 15). Although there are various hypotheses on how whole grain consumption may contribute to liver health, the exact mechanisms are as of yet unknown (11).

We previously performed a randomized, double-blind, parallel trial (16) in 50 overweight individuals and found that 12 weeks of 98 g/d refined wheat (RW) products resulted in a 49% relative increase in intrahepatic triglycerides (IHTG), while IHTG was not affected by whole-grain wheat (WGW) intervention. In the current study, we investigated the effects of the 12-week RW or WGW intervention on plasma levels of metabolites involved in lipid metabolism, i.e. acylcarnitines, trimethylamine-N-oxide

(TMAO), choline, betaine, bile acids, and other signaling lipids, in order to explore potential mechanisms that underlie the preventive effect of whole grain consumption on liver fat accumulation.

Materials and methods

Study design and participants

The current study is a post-hoc analysis of a 12-week, randomized, double-blind, parallel trial performed from January to July 2015 at Wageningen University and Research, the Netherlands (Figure 1). Details on study procedures have been reported in the original article (16). The study population consisted of men and postmenopausal women aged 45–70 years, with BMI 25–35 kg/m^2, mildly elevated plasma cholesterol concentrations (> 5 mmol/L), and habitual consumption of bread and cereals. Exclusion criteria were use of cholesterol-lowering medication, gluten intolerance, smoking, alcohol consumption > 21 glasses/week, > 5 kg weight change in the month prior to screening, and history of medical or surgical events that may affect the study outcome. Fifty subjects were included and randomized to the RW or WGW group, with stratification for gender, age, BMI, and cholesterol level.

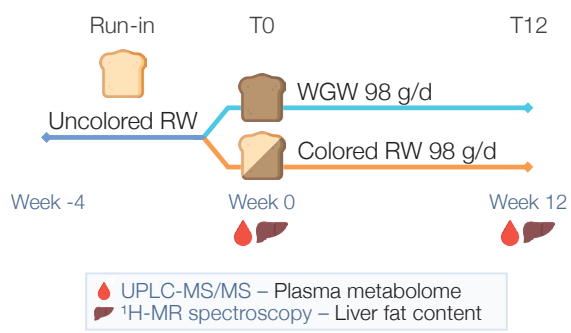

Figure 1. Study design. After a 4-week run-in period with uncolored refined wheat (RW) products, participants were randomized to 12 weeks of 98 g/d whole-grain wheat (WGW) products or RW products. In week 0 (T0) and 12 (T12), the plasma metabolome was measured using targeted ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and liver fat content was quantified using proton magnetic resonance spectroscopy (^{1}H-MRS).

Before the start of the intervention period, there was a 4-week run-in period with non-colored RW products to reduce variation of WGW intake in the study population at baseline. The primary outcome of the original study was the change in cardiometabolic risk factors in the WGW vs. RW group.

Intervention

RW or WGW products were provided to participants during the 12-week intervention period to replace their habitual intake: 100 g/d (four slices) of bread and 33.4 g/d of ready-to-eat-cereals, 98 g/d of RW or WGW flour in total. To match the appearance of the WGW products, RW products in the intervention period were colored using roasted wheat malt and caramelized sugar. RW and WGW products had comparable energy content and macronutrient composition, except for fiber content (RW 3.5 g fiber/100 g; WGW 7.8 g fiber/100 g) (16).

All participants were instructed to not consume additional whole-grain products during both the run-in period and the intervention period. Consumption of additional refined grain products was allowed in both groups. Compliance was evaluated by counting the weekly returned intervention product packages and measuring change in total plasma alkylresorcinol concentrations, which is a biomarker for whole-wheat grain intake.

Intrahepatic triglycerides and other liver parameters

IHTG was quantified by proton magnetic resonance spectroscopy (^{1}H-MRS) on a 3T whole-body scanner (Siemens, Munich, Germany) (16). Plasma levels of the ketone body β-hydroxybutyrate were measured by colorimetric assay. Plasma levels of the liver enzymes alanine transaminase (ALT), aspartate transaminase (AST), and γ-glutamyltransferase (γ-GT) were analyzed as described previously (16). Plasma concentrations of the acute-phase proteins serum amyloid A (SAA) and C-reactive protein were measured using immunoassays (16).

Fasting plasma parameters

Blood samples were drawn after an overnight fast. Plasma glucose, insulin, HbA1c, total cholesterol, HDL cholesterol, triglycerides (TG), and FFA were measured as described previously (16). The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated by dividing the product of fasting glucose (mmol/L) and insulin (mU/L) by 22.5.

Plasma metabolomics

Plasma metabolite levels were measured with two targeted ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) platforms by the Biomedical Metabolomics Facility Leiden (the Netherlands). The acylcarnitine platform covers acylcarnitines as well as betaine, choline, carnitine, and TMAO. The signaling lipid platform covers FFA, lysophospholipids, endocannabinoids, oxylipins, isoprostanes, prostaglandins, and bile acids. Details on the methods used for metabolomic analyses can be found in Supplementary Material. Metabolite levels are expressed as relative response ratios (target area/ISTD area; unit free) to appropriate internal

standards. After quality control correction, a total of 26 acylcarnitines, 61 signaling lipids including nine bile acids, and three other metabolites (betaine, choline, TMAO) complied with the acceptance criterium of RSDqc < 15%. Metabolites with ≥ 20% missing values per intervention group were removed from the dataset and metabolite measurements that fell below the limit of detection were imputed with half of the lowest observed level for this metabolite. The bile acid TCA was removed from the dataset due to 20% missing values (RW n = 5, WGW n = 5). Remaining missing values (n = 18 in total) were imputed with half of the lowest observed value for that respective metabolite.

Statistical analyses

Plasma metabolite levels were log2 transformed and autoscaled before analyses. Intervention effects on plasma metabolite levels were tested using ANCOVA with the post-intervention value as dependent variable and baseline value as covariate. Q-values corrected for a false discovery rate (FDR) of 0.05 were calculated using the Benjamin-Hochberg procedure. Within-group changes in individual metabolite levels upon the intervention were tested using paired t-tests.

To assess whether changes in metabolite levels upon the intervention were accompanied by changes in liver markers, we calculated Pearson correlation coefficients between changes in metabolite levels and changes in liver markers. We also assessed Pearson correlations between metabolite levels and liver markers at baseline. In addition, partial correlations with adjustment for age, gender, and BMI were calculated. Normality of the liver markers was assessed by visual inspection of residual Q-Q plots and they were log transformed if not normally distributed. All analyses were performed using SPSS version 25 software (IBM Corp.).

Results

All participants completed the 12-week intervention and plasma metabolite concentrations were measured in samples of all participants (RW n = 25, WGW n = 25). Baseline characteristics were comparable between the two intervention groups (Table 1).

As previously reported, compliance was between 99.5 and 100% based on return of intervention product packages, and 96% of participants could be correctly classified to either the RW or WGW group based on (change in) plasma alkylresorcinol concentrations (16).

Table 1. Baseline characteristics of the refined wheat (RW) and whole-grain wheat (WGW) groups.

	RW group (n = 25)	WGW group (n = 25)
Women, n (%)	9 (36.0%)	10 (40.0%)
Age, years	61 ± 6	61 ± 5
BMI, kg/m^2	27.6 ± 2.6	28.0 ± 2.1
HOMA-IR	1.9 [1.2, 2.5]	2.1 [1.2, 3.2]
HbA1c, mmol/mol	37.5 ± 2.3	36.1 ± 3.8
IHTG, %	2.5 [1.6, 6.9]	2.1 [1.7, 7.0]
Total cholesterol, mmol/L	5.8 ± 0.9	5.8 ± 0.7
HDL cholesterol, mmol/L	1.3 ± 0.3	1.3 ± 0.4
Triglycerides, mmol/L	1.5 [1.2, 1.9]	1.5 [1.0, 2.2]
Free fatty acids, mmol/L	0.5 ± 0.2	0.4 ± 0.1

Data are presented as mean ± SD or median [25th percentile, 75th percentile] if not normally distributed. BMI, body mass index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HbA1c, glycated hemoglobin; IHTG, intrahepatic triglycerides; HDL, high-density lipoprotein.

Whole-grain wheat vs. refined wheat effects on plasma acylcarnitines, bile acids, and signaling lipids

Plasma concentrations of five out of 52 signaling lipids were changed upon the WGW vs. RW intervention (Figure 2A and Supplementary Table 1). Lysophosphatidic acid C18:2 [LPA(18:2)] was increased upon intervention in both groups, but to a greater extent in the WGW group (log2 ratio mean ± SD: WGW: 0.79 ± 0.70, $p < 0.001$; RW: 0.33 ± 0.66, p = 0.018; WGW vs. RW, crude $p = 0.002$) (Figure 2B). Two lysophosphatidylethanolamine (LPE) species, LPE(18:1) and LPE(18:2), as well as platelet-activating factor C18:2 [PAF(18:2)] were increased in the WGW group, while they were not changed in the RW group (Figures 2C,D,F). Lysophosphatidylglycerol C20:3 [LPG(20:3)] was decreased in the WGW group (–0.36 ± 0.81, $p = 0.036$), and unchanged in the RW group (0.15 ± 0.77, $p = 0.32$; WGW vs. RW, crude $p = 0.028$) (Figure 2E). None of these differences remained statistically significant after FDR correction. We found no effects of WGW or RW intervention on plasma concentrations of betaine, choline, or TMAO, nor on the eight plasma bile acids and 26 plasma acylcarnitines that we quantified (Supplementary Table 1).

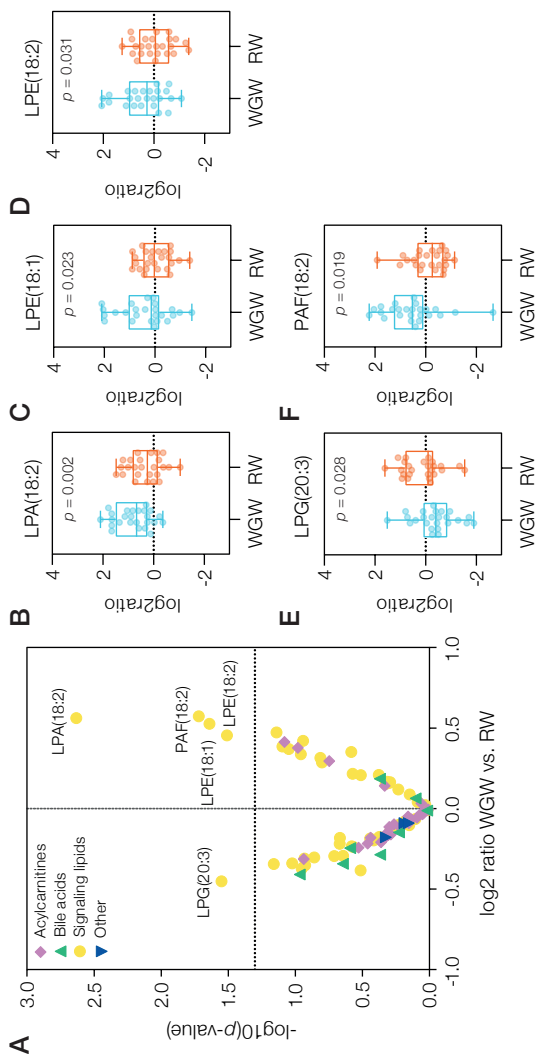

Figure 2. Effects of 12-week whole-grain wheat (WGW) vs. refined wheat (RW) intervention on plasma concentrations of 89 acylcarnitines, bile acids, and signaling lipids. **(A)** Volcano plot in which the differences in changes in individual metabolites between the WGW vs. RW group are plotted against –log 10(*p*-value), as tested by ANCOVA. The horizontal dotted line indicates $p = 0.05$. **(B–F)** Box plots of the change in plasma concentrations of lysophosphatidic acid C18:2 [LPA(18:2)] **(B)**, lysophosphatidylethanolamine C18:2 [LPE(18:2)] **(C)**, lysophosphatidylethanolamine C18:1 [LPE(18:1)] **(D)**, lysophosphatidylglycerol C20:3 [LPG(20:3)] **(E)**, and platelet-activating factor C18:2 [PAF(18:2)] **(F)** upon the WGW (blue) or RW (orange) intervention. The box plots represent the minimum, first quartile, median, third quartile, and maximum. Crude *p*-values are reported for the mean difference between the groups, as tested by ANCOVA.

Correlations between plasma metabolites and liver markers

As previously reported, 12 weeks of RW intervention resulted in a relative increase of 49% in IHTG, whereas 12 weeks of WGW intervention did not affect IHTG (16). To explore whether plasma levels of the five metabolites that were affected by WGW were related to liver health, we tested correlations between these metabolite levels and liver markers including IHTG, both in response to the 12-week intervention and at baseline. Change in IHTG was not correlated to change in plasma concentrations of any of the five metabolites (Figure 3B). Change in plasma LPE(18:1) was inversely correlated to change in CRP in the RW group ($r = -0.57$, $p = 0.004$), whereas change in plasma LPE(18:2) was inversely correlated to change in CRP in the WGW group ($r = -0.48$, $p = 0.01$). In addition, changes in plasma LPE(18:2) and LPG(20:3) were positively correlated to change in AST in the RW group ($r = 0.42$, $p = 0.04$; $r = 0.45$, $p = 0.02$), but not in the WGW group ($r = -0.16$, $p = 0.45$; $r = -0.05$, $p = 0.81$) (Figure 3B). Performing these correlation analyses in the complete study population while adjusting for intervention group strengthened the inverse correlations between changes in LPA(18:2), LPE(18:1), LPE(18:2) and CRP ($r = -0.29$, $p = 0.047$; $r = -0.37$, $p = 0.009$; $r = -0.41$, $p = 0.004$), and yielded similar results for the other correlations (data not shown).

At baseline, plasma LPA(18:2), LPE(18:1), and LPE(18:2) were inversely correlated to SAA and CRP ($r = -0.35$ to -0.39, $p < 0.05$) (Figure 3A). These correlations, however, were driven by data points from three participants, and exclusion of these data points resulted in a loss of significant correlations ($r = -0.08$ to -0.21, $p > 0.15$) (Supplementary Figure 1). Exclusion of these data points did not affect the correlations between changes in LPA(18:2), LPE(18:1), LPE(18:2) and change in CRP in the analyses in the complete study population or stratified for intervention group (data not shown). Plasma LPG(20:3) was positively correlated to IHTG ($r = 0.37$, $p = 0.02$), as well as to the liver enzymes ALT, AST, and γ-GT ($r = 0.29$–0.38, $p < 0.05$). LPE(18:2) was also positively correlated to γ-GT ($r = 0.32$, $p = 0.02$). PAF(18:2) levels were positively correlated to plasma levels of β-hydroxybutyrate ($r = 0.33$, $p = 0.019$) (Figure 3A). Adjustment for age, gender, and BMI annulled the correlation between change in LPE(18:2) and change in AST, but did not affect the other correlations.

Correlations between plasma betaine and choline and liver fat

We hypothesized that betaine and choline may be involved in WGW's protective effect on liver fat accumulation and therefore examined correlations between betaine and choline and IHTG, even though we did not find overall changes in plasma betaine and choline levels upon RW or WGW intervention. At baseline, IHTG was not correlated to plasma betaine or choline (Figures 4A,D). Upon intervention, change in IHTG was inversely correlated to change in plasma choline in the RW group ($r = -0.51$, $p = 0.03$) (Figure 4E) and to change in plasma betaine in the WGW group ($r = -0.47$, $p = 0.03$; Figure 4B), also after adjustment for age, gender, and BMI.

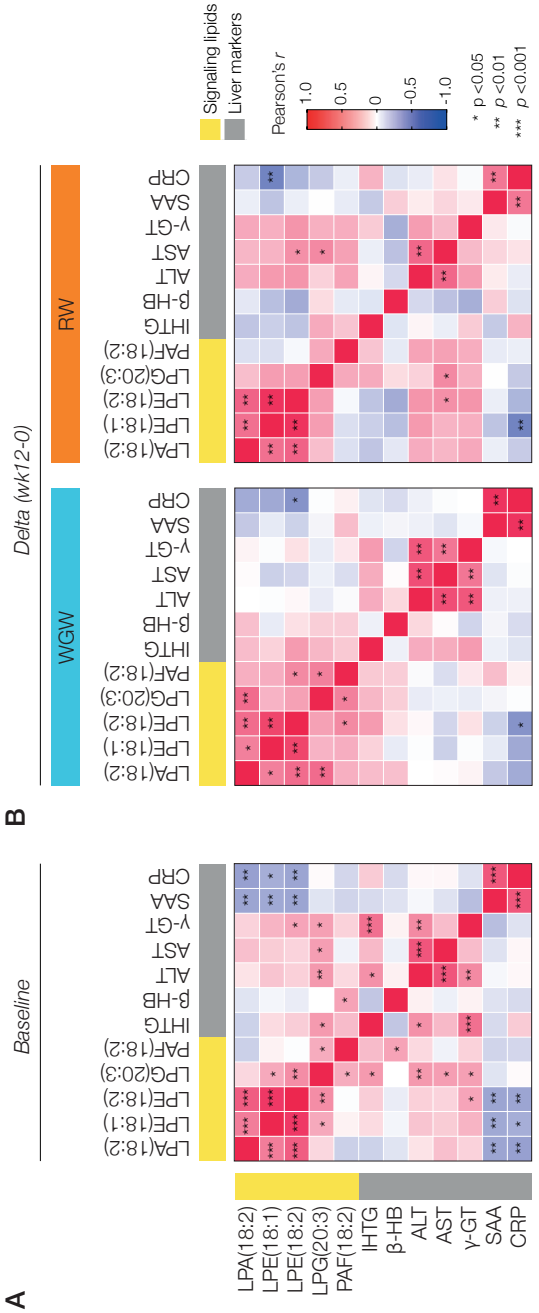

Figure 3. Correlation heatmap of Pearson correlations between plasma levels of LPA(18:2), LPE(18:1), LPE(18:2), LPG(20:3), and PAF(18:2), and liver markers including liver fat (IHTG). **(A)** Correlations at baseline. **(B)** Correlations between changes in LPA(18:2), LPE(18:1), LPE(18:2), LPG(20:3), and PAF(18:2) (expressed as log2 ratios) with changes in liver markers upon the 12-week whole-grain wheat (WGW) (left) or refined wheat (RW) (right) intervention. Asterisks indicate crude p-value < 0.05, < 0.01, or < 0.001. LPA(18:2), lysophosphatidic acid C18:2; LPE(18:1), lysophosphatidylethanolamine C18:1; LPE(18:2), lysophosphatidylethanolamine C18:2; LPG(20:3), lysophosphatidylglycerol C20:3; PAF(18:2), platelet-activating factor C18:2; IHTG, intrahepatic triglycerides; β-HB, β-hydroxybutyrate; ALT, alanine transaminase; AST, aspartate transaminase; γ-GT, γ-glutamyltransferase; SAA, serum amyloid A; CRP, C-reactive protein.

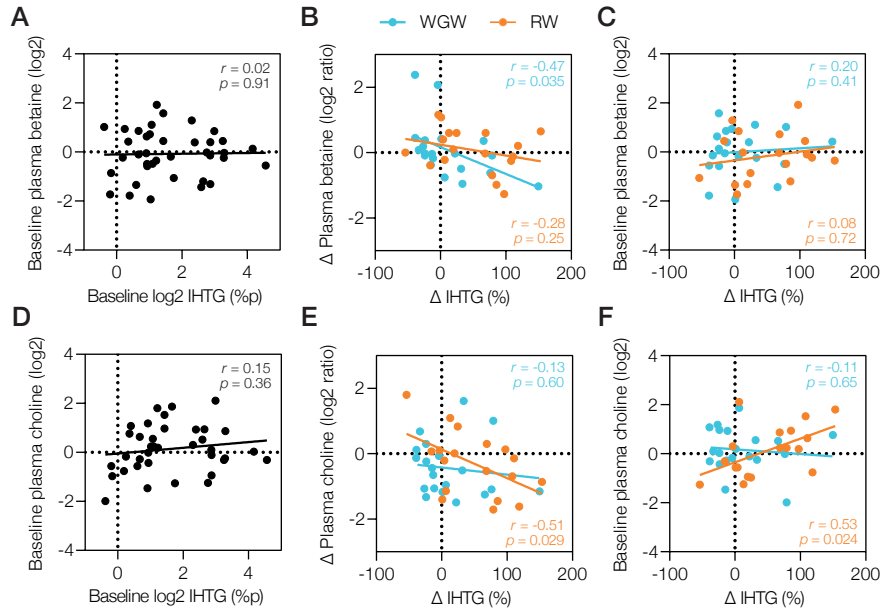

Figure 4. Plasma betaine and choline and effects of RW vs. WGW on IHTG. Pearson correlations between plasma betaine and IHTG at baseline **(A)**, change in plasma betaine and IHTG upon 12-week RW or WGW intervention **(B)**, baseline plasma betaine and change in IHTG **(C)**, plasma choline and IHTG at baseline **(D)**, change in plasma choline and IHTG upon 12-week RW or WGW intervention **(E)**, baseline plasma choline and change in IHTG **(F)**. Adjustment for age, gender, and BMI attenuated the correlation between baseline choline and change in IHTG (r = 0.42, p = 0.12).

We further explored whether the correlations of change in plasma choline and betaine levels with change in IHTG were dependent on baseline choline or betaine levels. Baseline betaine levels were not correlated to change in IHTG in the WGW group (r = 0.08, p = 0.72) (Figure 4C), nor did adjustment for baseline levels attenuate the correlation between change in betaine levels and change in IHTG (r = –0.47, p = 0.04). In the RW group, baseline choline levels were positively correlated to change in IHTG upon the intervention (r = 0.53, p = 0.02) (Figure 4F), and correction for baseline levels annulled the correlation with change in choline levels (r = –0.33, p = 0.19). Adjustment for age, gender, and BMI attenuated the correlation between baseline choline and change in IHTG (r = 0.42, p = 0.12).

Discussion

We previously showed that WGW consumption prevented liver fat accumulation, as compared to RW in overweight or obese individuals. Here, we investigated the effects of this 12-week WGW vs. RW intervention on plasma concentrations of a total of 89 acylcarnitines, bile acids, and signaling lipids with the aim to explore potential underlying mechanisms of the preventive effect of WGW consumption on liver fat accumulation. The WGW intervention affected plasma concentrations of five metabolites that belong to the (lyso)glycerophospholipid (GPL) class (17): WGW increased plasma platelet-activating factor C18:2 [PAF(18:2)], lysophosphatidylethanolamine C18:1 [LPE(18:1)], lysophosphatidylethanolamine C18:2 [LPE(18:2)], and lysophosphatidic acid C18:2 [LPA(18:2)], and decreased plasma lysophosphatidylglycerol C20:3 [LPG(20:3)], as compared to RW intervention. These results, however, were no longer statistically significant after FDR correction. The change in liver fat upon 12 weeks of RW or WGW was not accompanied by changes in levels of either of these five metabolites.

Whole grains have previously been reported to affect plasma levels of several GPLs. Compared to refined grains, whole grain intervention has been found to reduce plasma levels of the choline derivative glycerophosphocholine (GPC) (18), and increase plasma levels of various lysophosphatidylcholine (LPC) and phosphatidylcholine (PC) species (19, 20). To our knowledge, no prior studies have reported the effects of whole grain intervention on the specific plasma GPL that we measured.

WGW intervention increased circulating levels of PAF(18:2), while 12 weeks of RW did not affect PAF. PAF is a glycerophosphocholine that mediates a broad range of biological actions. It is produced by platelets, endothelial cells, and immune cells in response to various stimuli and is primarily known for its pro-inflammatory actions (21–23). Circulating PAF has been reported to be elevated in CVD and various inflammatory diseases in humans (24–26). Accordingly, the observed increase in plasma PAF upon WGW intervention may point toward increased inflammation, but this is unlikely since the WGW intervention actually tended to decrease the markers of systemic inflammation CRP and SAA, as we previously reported (16). Paradoxically, deficiency of the PAF receptor in mice fed a high-fat or high-carbohydrate diet has been found to impair metabolic health and increase liver fat accumulation, which indicates that PAF signaling may be required for maintenance of metabolic health in diet-induced obesity (27–32). The ramifications of the increase in plasma PAF levels upon WGW thus remain elusive.

In addition, WGW increased plasma LPE(18:1) and LPE(18:2), compared to RW. The physiological functions of LPEs are largely unknown (33). Individuals with NAFLD have been reported to have lower circulating LPE(18:1) and LPE(18:2) levels compared to healthy controls (34), and plasma LPE(18:1) and LPE(18:2) have been inversely associated with incident T2DM (35). We observed inverse correlations between change in these plasma LPEs and change in CRP upon the interventions, indicating that increases in these LPEs were accompanied by a reduction in systemic inflammation. Although no significant correlations with changes in liver fat were observed, the WGW-induced increase in plasma LPE(18:1) and LPE(18:2) may point toward a potential lead for the protective role of WGW on liver fat accumulation.

Both the WGW and RW interventions increased circulating LPA(18:2) levels, but WGW resulted in a larger increase. Circulating LPA is primarily generated by the adipose tissue-derived enzyme autotaxin, which hydrolyzes LPC and other lysophospholipids into LPA in the circulation (36). Serum autotaxin levels have been reported to be elevated in hepatic steatosis and (hepatic) inflammatory diseases (37–40). Although actual LPA levels were not measured in the majority of these cross-sectional studies, it can be expected that LPA levels are similarly elevated in these conditions, since serum autotaxin levels are strongly positively correlated to plasma LPA levels (37, 41). In mice, heterozygous knockout of ATX, resulting in 50% reduced circulating LPA levels, has been reported to mitigate high-fat diet-induced liver fat accumulation and inflammation (42). In contrast to these findings, we observed an increase in plasma LPA(18:2) and prevention of liver fat accumulation upon WGW consumption, as compared to RW, as well as an inverse correlation between change in plasma LPA(18:2) and change in CRP, with increases in LPA(18:2) thus being accompanied by a reduction in systemic inflammation. Hence, LPA seems to be implicated in inflammation and hepatic lipid metabolism, but the cause and interpretation of the increases in plasma levels we observed after WGW intervention remain as of yet unclear.

Compared to the RW intervention, the WGW intervention decreased plasma concentrations of LPG(20:3). LPG is a precursor of de novo synthesis of phosphatidylglycerol, a phospholipid that is mainly abundant in lung surfactant (33, 43). Very little is known about its biological actions (33, 43). At baseline, plasma LPG(20:3) was positively correlated to IHTG and the liver enzymes ALT, AST, and γ-GT, but the decrease in LPG(20:3) upon the WGW intervention was not accompanied by change in liver fat nor liver enzymes. It thus appears that plasma LPG might be related to liver health and/or function, although the nature of the association is unclear since we did not observe parallel changes in LPG and liver fat upon WGW. This lack of correlation may also be partly due to limited power after stratification for intervention group. Future studies with large sample size are required to clarify the potential role of LPG in liver fat accumulation.

We hypothesized that choline and betaine may be involved in the protective effect of WGW on liver fat accumulation given their roles in TG secretion from the liver into the circulation as VLDL and hepatic one-carbon metabolism, respectively. Plasma choline was not significantly different between WGW vs. RW intervention,

which is in line with findings from a cross-over trial that tested 8-week interventions with either 50 g/1,000 kcal whole grains or refined grains in 33 overweight or obese individuals (44). We also did not observe changes in plasma betaine upon WGW or RW. Various other studies did report increased plasma betaine levels after whole grain intervention (45–47), although not all (18, 44, 48). This incongruency seems to arise primarily from differences in whole grain dose: the studies that reported increases in betaine levels used 200–485 g/d of whole-grain cereals or bread (45–47), which is considerably more than the 133 g/d in our study.

Interestingly, we did observe that an increase in plasma betaine upon the WGW intervention was correlated to a reduction in liver fat. The steatosis-lowering potential of betaine, however, remains controversial since the beneficial effects of betaine supplementation that have been observed in animal studies (14) have only been reproduced in humans in a small pilot study in 10 NASH patients, (49) and not in recent clinical trials in individuals with NAFLD or prediabetes (50, 51). It could be speculated that the effects of increasing betaine intake with betaine-rich foods or supplements on liver fat may depend on individual characteristics such as sex, BMI, health status, habitual diet, and other factors, since such factors appear to mediate the effects of betaine on cardiovascular risk factors (52) and the same may be the case for effects of betaine on liver fat.

Strengths of this study include the relatively long intervention period of 12 weeks, which enabled us to study longer-term rather than acute effects of WGW and RW consumption. In addition, we included a 4-week run-in period with RW for all participants to reduce variation in the study population at the start. Both researchers and participants were blinded to the intervention by coloring RW products to match the appearance of WGW products. Compliance to the intervention based on recall of empty product packages and plasma alkylresorcinol levels was high and all participants completed the study.

This study may be limited by its relatively small sample size, which was originally determined to detect changes in plasma cholesterol levels. Given the large inter-individual variation in plasma metabolite concentrations—both at baseline and in response to a 12-week diet—we may have missed effects of the WGW or RW intervention on plasma metabolite levels due to insufficient power. In addition, the five metabolites that we identified to be differentially affected by WGW compared to RW intervention in this explorative study were no longer statistically significantly different after FDR correction. There is a possibility that these findings are chance findings and they need to be confirmed in larger studies.

Conclusion

In conclusion, in this post-hoc analysis of a double-blind, randomized controlled trial investigating the effects of 12 weeks of WGW or RW intervention on plasma acylcarnitines, bile acids, and signaling lipids in middle-aged, overweight adults, we observed that plasma concentrations of five signaling lipids involved in glycerophospholipid metabolism were altered upon WGW as compared to RW intervention, but these changes did not remain statistically significant after FDR correction. The changes in plasma concentrations of these five signaling lipids upon the intervention were not correlated to changes in liver fat, which makes these metabolites less likely candidates to be involved in the mechanisms underlying the protective effect of WGW consumption or detrimental effect of RW consumption on liver fat accumulation.

Data availability statement

The datasets presented in this article are not readily available because participants did not provide consent for their data to be shared publicly. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Wageningen University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

DE, SW, and LA designed the study. SS and DE coordinated and performed the execution of the trial. LA supervised execution of the trial. AG analyzed the data and wrote the draft manuscript. GG and LA critically revised the draft. All authors read and approved the final manuscript.

Funding

This study was supported by the public-private partnership “Combining innovation with tradition: improving resilience with essential nutrients and whole wheat bread,” financed by Topsector Agri and Food (TKI-AF 12083) and TNO roadmap Nutrition and Health and co-funded by Cereal Partners Worldwide, the Dutch Bakery Center, and GoodMills Innovation GmbH. The metabolomics analyses were supported by the ZonMW Enabling Technologies Hotels application 40-43500-98-4029. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Acknowledgments

We thank the volunteers for their participation in the study and Roland Hangelbroek for his advice on pre-treatment of the metabolomics data.

Conflict of interest

This study received funding from Topsector Agri and Food, TNO roadmap Nutrition and Health, Cereal Partners Worldwide, the Dutch Bakery Center, GoodMills Innovation GmbH, and ZonMW. The funders had the following involvement with the study: sponsorship.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

Metabolomics Methods

Plasma metabolite levels were measured with two targeted (UP)LC-MS/MS platforms by the Biomedical Metabolomics Facility Leiden (the Netherlands). All samples were randomized and run in two batches which included calibration lines, quality control (QC) samples and blanks. QC samples were analyzed every 10 samples and were used to assess data quality and to correct for instrument response. Blanks were used to check for background signal compared to the study samples.

The acylcarnitine platform covers acylcarnitines as well as betaine, choline, carnitine, and trimethyl-amine-n-oxide. Ten µL of each sample was spiked with an internal standard solution. Proteins were precipitated by the addition of methanol, after which the supernatant was transferred to an autosampler vial. The vials were transferred to an autosampler tray and cooled to 10°C until the injection. One µL of the sample mixture was injected into the UPLCMS/MS. Chromatographic separation was achieved by UPLC (Agilent 1290, San Jose, CA, USA) on an Accq-Tag Ultra column (Waters). The UPLC was coupled to electrospray ionization on a triple quadrupole mass spectrometer (Agilent 6460, San Jose, CA, USA). Analytes were detected in the positive ion mode and monitored in Multiple Reaction Monitoring (MRM) using nominal mass resolution. Acquired data were evaluated using Agilent MassHunter Quantitative Analysis software (Agilent, Version B.05.01), by integration of assigned MRM peaks and normalization using proper internal standards. The closest-eluting internal standard was employed. In-house developed algorithms were applied using the pooled QC samples to compensate for shifts in the sensitivity of the mass spectrometer over the batches.

The signaling lipid platform covers free fatty acids, lysophospholipids, endocannabinoids, oxylipins, isoprostanes, prostaglandins, and bile acids. The signaling lipids platform is divided in two chromatographic methods: low and high pH. In the low pH method, isoprostanes, prostaglandins, nitro-fatty acids, lysosphingolipids, endocannabinoids, and bile acids are analyzed. The high pH method covers lysosphingolipids, lysophosphatidic acids, lysophosphatidylglycerol, lyso-phosphatidylinositol, lyso-phosphatidyserine, lysophosphatidylethanolamines, cyclic-phosphatidic acids and fatty acids. Each sample was spiked with antioxidant and internal standard solution. The extraction of the compounds was performed via liquid-liquid extraction. To extract the analytes from the aqueous phase, butanol and methyl tert-butyl ether were used. After collection, the organic phase was concentrated by first drying and then reconstituted in a smaller volume. After reconstitution, the extract was transferred into amber autosampler vials and used for high and low pH injection. A Shimadzu system formed by three high pressure pumps (LC-30AD), controller (CBM-20Alite), auto sampler (SIL-30AC) and an oven (CTO-30A) from Shimadzu Benelux, was coupled online with a LCMS-8050 triple quadrupole mass spectrometer (Shimadzu) for high pH measurements. A LCMS-8060 triple quadrupole mass spectrometer (Shimadzu) was coupled to the Shimadzu system for low pH measurements. Both systems were operated using LabSolutions data acquisition software (Version 5.89, Shimadzu). An Acquity UPLC BEH C18 column (Waters) was used to measure the samples in the low pH method. For the high pH method, a Kinetex EVO column by Phenomenex was used. The triple quadrupole mass spectrometer was used in polarity switching mode and all analytes were monitored in dynamic MRM. The acquired data was evaluated using LabSolutions Insight software (Version 3.3, Shimadzu), by integration of assigned MRM peaks and normalization using accordingly selected internal standards. When available, a deuterated version of the target compound was used as internal standard. For the other compounds, the closest-eluting internal standard was employed. Blank samples were used to check blank levels.

Supplementary Table 1. Effects of 12 weeks of refined wheat (RW) or whole-grain wheat (WGW) intervention on plasma acylcarnitines, bile acids, and signaling lipids.

	Refined wheat group (n = 25)			Whole-grain wheat group (n = 25)			RW vs. WGW	
	Baseline[a]	log2 ratio wk12-0	*p*-value[b]	Baseline[a]	log2 ratio wk12-0	*p*-value[b]	*p*-value[c]	FDR *q*-value[d]
Acylcarnitines								
2-methylbutyroylcarnitine	0.125 ± 0.925	0.091 ± 0.633	0.479	-0.253 ± 1.133	0.165 ± 0.698	0.249	0.861	0.946
Acetylcarnitine	0.011 ± 1.127	0.143 ± 1.105	0.524	-0.221 ± 1.085	0.276 ± 1.213	0.267	0.886	0.950
Butyrylcarnitine	0.049 ± 1.071	0.117 ± 0.385	0.142	-0.143 ± 0.864	0.072 ± 0.488	0.467	0.691	0.867
Carnitine	-0.148 ± 1.024	0.385 ± 0.701	0.011	-0.064 ± 1.079	0.040 ± 0.865	0.821	0.116	0.605
Decanoylcarnitine	0.494 ± 0.890	-0.635 ± 0.666	<0.001	0.111 ± 1.048	-0.575 ± 0.800	0.001	0.730	0.855
Decenoylcarnitine	0.479 ± 0.908	-0.487 ± 0.756	0.004	-0.012 ± 0.930	-0.448 ± 0.893	0.019	0.607	0.806
Dodecenoylcarnitine	0.554 ± 0.939	-0.628 ± 0.810	0.001	0.072 ± 0.940	-0.626 ± 0.945	0.003	0.345	0.830
Hexadecenoylcarnitine	0.162 ± 0.882	-0.178 ± 0.770	0.259	0.086 ± 1.031	-0.317 ± 0.999	0.125	0.491	0.809
Hexanoylcarnitine	0.251 ± 0.915	-0.174 ± 0.561	0.133	-0.111 ± 0.935	-0.106 ± 0.767	0.497	0.866	0.940
Isobutyrylcarnitine	0.037 ± 1.124	0.006 ± 0.476	0.951	-0.054 ± 1.120	0.028 ± 0.644	0.831	0.962	0.984
Isovalerylcarnitine	-0.382 ± 0.936	0.896 ± 0.737	<0.001	-0.396 ± 0.862	0.659 ± 1.010	0.003	0.295	0.773
Lauroylcarnitine	0.495 ± 0.849	-0.578 ± 0.783	0.001	0.065 ± 1.095	-0.541 ± 0.921	0.007	0.488	0.819
Linoleylcarnitine	-0.126 ± 0.889	-0.135 ± 0.738	0.368	0.174 ± 1.136	0.041 ± 0.944	0.830	0.179	0.691
Malonylcarnitine	0.047 ± 1.062	0.065 ± 1.060	0.761	-0.027 ± 0.901	-0.105 ± 1.068	0.629	0.435	0.841
Myristoilcarnitine	0.155 ± 0.895	-0.245 ± 0.795	0.137	0.171 ± 1.038	-0.407 ± 0.816	0.020	0.471	0.856
Nonaylcarnitine	0.016 ± 1.016	0.032 ± 0.419	0.703	-0.002 ± 1.077	-0.060 ± 0.739	0.687	0.543	0.779
Octanoylcarnitine	0.452 ± 0.887	-0.536 ± 0.631	<0.001	0.031 ± 1.024	-0.432 ± 0.783	0.011	0.979	0.979
Octenoylcarnitine	0.023 ± 1.013	0.070 ± 0.604	0.565	-0.200 ± 1.077	0.283 ± 0.859	0.112	0.462	0.875
Oleylcarnitine	-0.119 ± 0.894	-0.198 ± 0.939	0.303	0.235 ± 1.229	-0.034 ± 1.095	0.877	0.104	0.774
Palmitoylcarnitine	-0.143 ± 0.902	0.081 ± 0.751	0.597	0.145 ± 1.119	-0.083 ± 0.960	0.670	0.804	0.906
Propionylcarnitine	-0.210 ± 1.289	0.403 ± 0.911	0.037	-0.072 ± 0.825	0.162 ± 0.752	0.292	0.363	0.829
Stearoylcarnitine	-0.075 ± 0.874	-0.165 ± 0.939	0.389	0.066 ± 1.142	0.183 ± 0.937	0.339	0.083	0.818
Tetradecadienylcarnitine	0.461 ± 0.841	-0.580 ± 0.846	0.002	0.127 ± 1.038	-0.594 ± 1.022	0.008	0.479	0.835
Tetradecenoylcarnitine	0.363 ± 0.793	-0.470 ± 0.798	0.007	0.137 ± 1.077	-0.531 ± 1.082	0.022	0.514	0.776
Tiglylcarnitine	0.218 ± 0.799	-0.034 ± 0.708	0.812	-0.239 ± 1.110	0.076 ± 0.921	0.682	0.899	0.953
Valerylcarnitine	0.096 ± 1.158	0.139 ± 0.564	0.230	-0.221 ± 0.913	0.112 ± 0.718	0.445	0.501	0.811
Bile acids								
Cholic acid	-0.191 ± 0.983	0.023 ± 0.956	0.905	0.135 ± 0.988	0.088 ± 0.787	0.581	0.427	0.863
Glycochenodeoxycholic acid	-0.136 ± 0.620	0.203 ± 0.611	0.110	0.107 ± 0.869	-0.146 ± 1.221	0.555	0.226	0.746
Glycodeoxycholic acid	-0.233 ± 0.868	0.398 ± 0.781	0.018	0.078 ± 0.938	-0.088 ± 0.983	0.657	0.108	0.736
Glycolithocholic acid	-0.151 ± 0.995	0.340 ± 0.990	0.098	-0.090 ± 1.072	0.142 ± 1.515	0.642	0.591	0.810
Glycoursodeoxycholic acid	-0.304 ± 0.701	0.601 ± 1.751	0.099	-0.070 ± 0.578	0.147 ± 0.627	0.252	0.428	0.847
Lithocholic acid sulphate	-0.180 ± 1.211	0.195 ± 0.978	0.329	0.169 ± 0.790	-0.172 ± 0.617	0.176	0.256	0.786

Supplementary Table 1. Continued.

	Refined wheat group (n = 25)			Whole-grain wheat group (n = 25)			RW vs. WGW	
	Baseline[a]	log2 ratio wk12-0	*p*-value[b]	Baseline[a]	log2 ratio wk12-0	*p*-value[b]	*p*-value[c]	FDR *q*-value[d]
Bile acids								
Taurodeoxycholic acid	-0.171 ± 0.994	0.158 ± 0.717	0.280	0.023 ± 1.033	0.139 ± 1.088	0.528	0.788	0.899
Taurohyodeoxycholic acid	-0.156 ± 0.953	0.190 ± 0.876	0.289	0.007 ± 1.020	0.108 ± 1.037	0.606	0.962	0.995
Signaling lipids								
12,13-dihydroxy-9Z-octadecenoic acid	-0.028 ± 0.811	-0.238 ± 0.492	0.024	0.327 ± 1.072	-0.360 ± 0.720	0.020	0.654	0.844
Adrenic acid	0.023 ± 1.170	0.131 ± 0.707	0.364	0.034 ± 0.706	-0.245 ± 0.986	0.226	0.112	0.662
Anandamide	0.041 ± 1.123	-0.099 ± 0.779	0.532	0.279 ± 1.004	-0.540 ± 0.878	0.005	0.095	0.767
Cyclic-Lysophosphatidic acid (18:0)	-0.040 ± 0.646	-0.294 ± 1.375	0.295	0.162 ± 0.658	0.051 ± 0.620	0.683	0.261	0.775
Cortisol	0.164 ± 0.943	-0.169 ± 0.740	0.264	-0.167 ± 0.984	0.174 ± 1.145	0.456	0.419	0.909
Docosahexaenoic acid	0.196 ± 0.927	-0.212 ± 0.546	0.064	0.100 ± 1.090	-0.379 ± 0.517	0.001	0.215	0.736
Docosapentaenoic acid	0.131 ± 0.997	-0.248 ± 0.587	0.045	0.189 ± 0.866	-0.392 ± 0.873	0.034	0.521	0.760
Linoleoyl ethanolamide	0.004 ± 1.000	-0.318 ± 0.891	0.087	0.097 ± 0.877	0.115 ± 1.072	0.596	0.072	0.920
Linoleic acid	0.220 ± 0.931	-0.198 ± 0.584	0.103	0.038 ± 1.036	-0.317 ± 0.998	0.125	0.421	0.892
Lysophosphatidic acid (14:0)	-0.332 ± 1.152	0.670 ± 1.068	0.004	-0.122 ± 1.082	0.239 ± 0.774	0.135	0.117	0.577
Lysophosphatidic acid (16:0)	-0.497 ± 0.693	0.527 ± 0.693	0.001	0.055 ± 1.089	0.357 ± 1.016	0.092	0.932	0.976
Lysophosphatidic acid (16:1)	-0.159 ± 0.972	0.237 ± 0.668	0.088	-0.010 ± 1.055	0.100 ± 0.712	0.488	0.588	0.818
Lysophosphatidic acid (18:1)	-0.569 ± 0.648	0.293 ± 0.699	0.047	0.186 ± 1.030	0.474 ± 0.756	0.004	0.080	0.890
Lysophosphatidic acid (18:2)	-0.448 ± 0.781	0.331 ± 0.655	0.018	-0.111 ± 1.106	0.787 ± 0.691	0.000	0.002	0.207
Lysophosphatidic acid (22:6)	-0.153 ± 0.983	0.085 ± 0.685	0.539	-0.017 ± 1.030	0.253 ± 0.823	0.137	0.307	0.759
Lysophosphatidylethanolamine (16:0)	-0.138 ± 0.894	-0.047 ± 0.561	0.681	0.051 ± 1.012	0.221 ± 0.971	0.266	0.153	0.650
Lysophosphatidylethanolamine (16:1)	-0.088 ± 1.045	0.099 ± 0.699	0.484	0.054 ± 0.984	-0.030 ± 0.850	0.861	0.672	0.854
Lysophosphatidylethanolamine (18:0)	-0.114 ± 0.844	-0.039 ± 0.567	0.735	0.006 ± 1.171	0.256 ± 0.984	0.205	0.110	0.697
Lysophosphatidylethanolamine (18:1)	-0.241 ± 0.885	-0.022 ± 0.614	0.861	0.044 ± 0.975	0.417 ± 0.988	0.045	0.023	0.680
Lysophosphatidylethanolamine (18:2)	-0.144 ± 0.970	-0.013 ± 0.705	0.927	-0.054 ± 1.055	0.410 ± 0.876	0.028	0.031	0.550
Lysophosphatidylethanolamine (18:3)	-0.239 ± 1.007	0.185 ± 1.160	0.434	0.049 ± 1.097	0.195 ± 0.783	0.226	0.481	0.822
Lysophosphatidylethanolamine (20:3)	-0.092 ± 0.904	0.012 ± 0.843	0.945	0.022 ± 1.069	0.129 ± 0.960	0.508	0.501	0.797
Lysophosphatidylethanolamine (20:4)	0.015 ± 0.902	-0.202 ± 0.626	0.119	0.000 ± 1.082	0.172 ± 0.951	0.374	0.089	0.794
Lysophosphatidylethanolamine (20:5)	-0.139 ± 1.006	0.160 ± 0.744	0.293	-0.058 ± 1.184	0.235 ± 1.107	0.299	0.584	0.825
Lysophosphatidylethanolamine (22:4)	-0.060 ± 0.938	0.013 ± 0.665	0.920	-0.024 ± 1.079	0.155 ± 0.902	0.400	0.471	0.873
Lysophosphatidylethanolamine (22:5)	-0.081 ± 0.958	-0.017 ± 0.766	0.913	0.041 ± 1.010	0.097 ± 1.096	0.662	0.508	0.780
Lysophosphatidylethanolamine (22:6)	0.051 ± 0.913	-0.184 ± 0.530	0.095	0.021 ± 1.137	0.040 ± 0.896	0.824	0.267	0.742
Lysophosphatidylglycerol (16:0)	-0.063 ± 0.913	0.050 ± 0.750	0.740	0.109 ± 1.059	-0.142 ± 0.846	0.410	0.506	0.790
Lysophosphatidylglycerol (16:1)	0.056 ± 1.189	0.016 ± 0.864	0.927	0.109 ± 0.860	-0.345 ± 0.538	0.004	0.069	1.024
Lysophosphatidylglycerol (18:0)	-0.067 ± 0.907	0.103 ± 0.562	0.368	-0.027 ± 1.043	0.084 ± 0.925	0.653	0.964	0.975
Lysophosphatidylglycerol (18:1)	0.038 ± 0.871	-0.123 ± 0.711	0.396	0.291 ± 1.005	-0.535 ± 0.875	0.005	0.118	0.553

Supplementary Table 1. Continued.

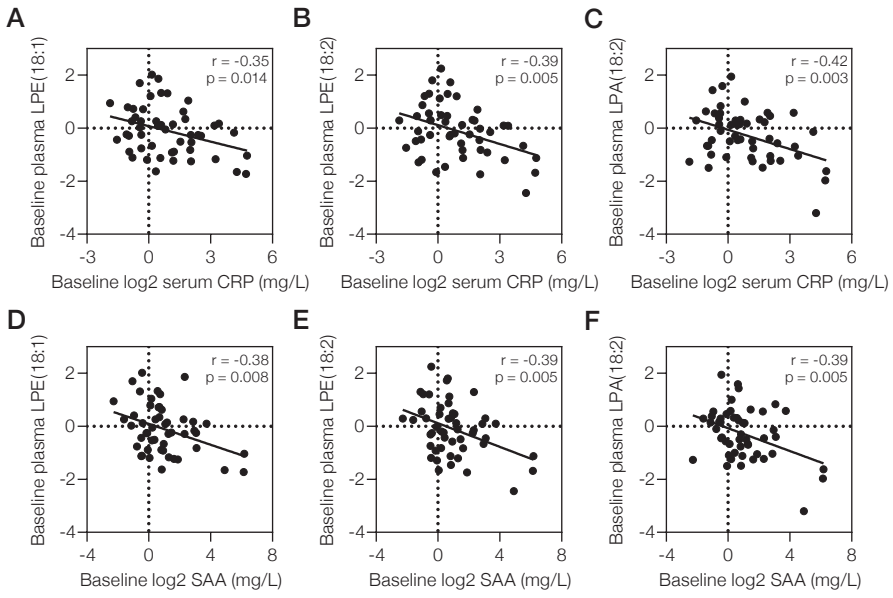
	Refined wheat group (n = 25)			Whole-grain wheat group (n = 25)			RW vs. WGW	
	Baseline[a]	log2 ratio wk12-0	*p*-value[b]	Baseline[a]	log2 ratio wk12-0	*p*-value[b]	*p*-value[c]	FDR *q*-value[d]
Signaling lipids								
Lysophosphatidylglycerol (18:2)	-0.080 ± 1.031	-0.042 ± 0.753	0.782	0.240 ± 0.849	-0.278 ± 0.774	0.085	0.423	0.875
Lysophosphatidylglycerol (20:3)	-0.036 ± 1.066	0.154 ± 0.768	0.324	0.139 ± 1.019	-0.360 ± 0.811	0.036	0.028	0.625
Lysophosphatidylglycerol (20:4)	0.041 ± 1.108	-0.152 ± 0.729	0.308	0.301 ± 0.819	-0.533 ± 0.943	0.009	0.195	0.723
Lysophosphatidylinositol (16:0)	0.009 ± 1.030	0.234 ± 0.770	0.141	-0.153 ± 1.002	0.054 ± 0.817	0.745	0.263	0.755
Lysophosphatidylinositol (16:1)	0.022 ± 1.113	0.190 ± 0.749	0.217	-0.073 ± 0.887	-0.087 ± 0.769	0.579	0.138	0.614
Lysophosphatidylinositol (18:0)	0.176 ± 0.880	0.075 ± 0.701	0.598	-0.215 ± 1.100	0.004 ± 0.806	0.981	0.360	0.844
Lysophosphatidylinositol (18:1)	-0.136 ± 1.058	0.221 ± 0.686	0.121	-0.099 ± 1.095	0.249 ± 0.747	0.109	0.815	0.907
Lysophosphatidylinositol (18:2)	-0.165 ± 0.869	0.310 ± 0.846	0.080	-0.020 ± 1.117	0.061 ± 0.871	0.727	0.384	0.853
Lysophosphatidylinositol (20:4)	0.070 ± 1.019	0.038 ± 0.898	0.835	-0.103 ± 0.904	0.026 ± 0.850	0.878	0.784	0.906
Lysophosphatidylinositol (22:4)	0.132 ± 1.086	-0.262 ± 0.963	0.186	0.034 ± 0.836	-0.069 ± 0.859	0.692	0.515	0.764
Lysophosphatidylserine (18:0)	-0.067 ± 0.904	-0.135 ± 0.605	0.277	0.084 ± 1.149	0.100 ± 0.917	0.591	0.157	0.637
Lysophosphatidylserine (20:4)	0.034 ± 1.218	-0.176 ± 1.367	0.525	-0.126 ± 0.858	0.361 ± 0.987	0.080	0.114	0.634
Oleic acid	0.187 ± 0.955	-0.166 ± 0.506	0.115	-0.083 ± 0.816	-0.042 ± 1.040	0.842	0.712	0.845
Osbond acid	0.213 ± 1.126	-0.263 ± 0.748	0.091	0.194 ± 0.704	-0.551 ± 1.038	0.014	0.227	0.722
Platelet activating factor (18:2)	-0.026 ± 1.019	-0.102 ± 0.729	0.491	-0.193 ± 0.940	0.540 ± 1.069	0.019	0.019	0.849
Prostaglandin F2α	0.044 ± 0.977	-0.106 ± 0.949	0.582	0.128 ± 0.973	-0.237 ± 0.553	0.042	0.603	0.813
Sphingosine-1-phosphate (16:1)	0.154 ± 0.348	0.053 ± 0.167	0.126	0.007 ± 0.366	-0.376 ± 1.794	0.305	0.306	0.778
Sphingosine-1-phosphate (18:0)	-0.143 ± 1.014	0.096 ± 0.929	0.609	0.169 ± 0.886	-0.148 ± 1.108	0.511	0.704	0.847
Sphingosine-1-phosphate (18:1)	-0.208 ± 1.184	0.193 ± 0.953	0.322	0.126 ± 0.817	-0.030 ± 0.805	0.856	0.695	0.859
Sphingosine-1-phosphate (18:2)	-0.169 ± 1.069	0.149 ± 0.663	0.273	0.162 ± 0.935	-0.133 ± 0.595	0.275	0.214	0.762
Virodhamine	0.039 ± 0.911	-0.044 ± 0.844	0.795	0.141 ± 1.108	-0.317 ± 0.797	0.058	0.271	0.730
Other								
Betaine	-0.117 ± 1.058	0.233 ± 0.717	0.117	-0.064 ± 0.910	0.128 ± 0.767	0.414	0.646	0.846
Choline	0.239 ± 1.057	-0.294 ± 1.022	0.163	0.117 ± 0.871	-0.418 ± 0.842	0.020	0.476	0.848
Trimethylamine N-oxide	-0.020 ± 0.898	0.167 ± 0.985	0.404	-0.130 ± 1.194	0.134 ± 0.977	0.501	0.700	0.854

[a] Values are log2-transformed and autoscaled relative response ratios

[b] Within-group changes in individual metabolite levels upon the intervention were tested using paired t-tests

[c] Between-group differences in effects on plasma metabolite levels were tested using ANCOVA with the post-intervention value as dependent variable and baseline value as covariate

[d] Q-values corrected for a false discovery rate (FDR) of 0.05 were calculated using the Benjamin-Hochberg procedure



Supplementary Figure 1. Scatter plots with Pearson correlations between baseline plasma LPE(18:1), LPE(18:2), LPA(18:2) and serum C-reactive protein (CRP) (**A-C**), and LPE(18:1), LPE(18:2), LPA(18:2) and serum amyloid A concentrations (SAA) (**D-F**). These correlations were driven by data points from three participants, and exclusion of these data points resulted in a loss of significant correlations (r = -0.08 to -0.21, $p > 0.15$).

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The PERSonalized glucose Optimization through Nutritional intervention (PERSON) study: rationale, design and preliminary screening results

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Frontiers in Nutrition **2021**, Jun 30;*8*:694568

Abstract

Background: It is well-established that the etiology of type 2 diabetes differs between individuals. Insulin resistance (IR) may develop in different tissues, but the severity of IR may differ in key metabolic organs such as the liver and skeletal muscle. Recent evidence suggests that these distinct tissue-specific IR phenotypes may also respond differentially to dietary macronutrient composition with respect to improvements in glucose metabolism.

Objective: The main objective of the PERSON study is to investigate the effects of an optimal vs. suboptimal dietary macronutrient intervention according to tissue-specific IR phenotype on glucose metabolism and other health outcomes.

Methods: In total, 240 overweight/obese (BMI 25 – 40 kg/m^2) men and women (age 40 – 75 years) with either skeletal muscle insulin resistance (MIR) or liver insulin resistance (LIR) will participate in a two-center, randomized, double-blind, parallel, 12-week dietary intervention study. At screening, participants undergo a 7-point oral glucose tolerance test (OGTT) to determine the hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI), classifying each participant as either "No MIR/LIR," "MIR," "LIR," or "combined MIR/LIR." Individuals with MIR or LIR are randomized to follow one of two isocaloric diets varying in macronutrient content and quality, that is hypothesized to be either an optimal or suboptimal diet, depending on their tissue-specific IR phenotype (MIR/LIR). Extensive measurements in a controlled laboratory setting as well as phenotyping in daily life are performed before and after the intervention. The primary study outcome is the difference in change in disposition index, which is the product of insulin sensitivity and first-phase insulin secretion, between participants who received their hypothesized optimal or suboptimal diet.

Results: The PERSON study is one of the first randomized clinical trials in the field of precision nutrition to test effects of a more personalized dietary intervention based on IR phenotype. The results of the PERSON study will contribute knowledge on the effectiveness of targeted nutritional strategies to the emerging field of precision nutrition, and improve our understanding of the complex pathophysiology of whole body and tissue-specific IR. Clinical trial registration: [www.ClinicalTrials.gov], identifier [NCT03708419].

Introduction

The prevalence of overweight and related metabolic disturbances, including impaired glucose homeostasis, is rising at an alarming rate, thereby increasing the risk for type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (1). Dietary modulation can effectively lower blood glucose levels and reduce the risk of chronic metabolic diseases, independent of weight loss (2, 3). Interestingly, there is great heterogeneity in individuals' metabolic response to dietary interventions (4, 5). Part of this heterogeneity may be attributed to differences in adherence, but recent findings of large inter-individual variation in postprandial responses to standardized meals indicate that individuals actually respond differently to food (6, 7). This inter-individual variation in response to food has complex underpinnings that include biological (including genetic), environmental, and lifestyle factors, and may partly explain the differential metabolic impact of dietary interventions (4–9).

Whole-body insulin resistance (IR) reflects defective insulin action in tissues such as skeletal muscle, liver, adipose tissue, gut and brain, and is a major risk factor for T2DM and CVD. IR can develop concurrently in different tissues, but the severity of IR may vary between tissues (10, 11). Individuals may, for example, have IR predominantly in the liver or skeletal muscle (10). Liver insulin resistance (LIR) is manifested by impaired insulin-mediated suppression of hepatic glucose production (HGP), while muscle insulin resistance (MIR) is characterized by decreased insulin-mediated glucose disposal (11). The gold-standard method to quantify LIR and MIR is the two-step hyperinsulinemic-euglycemic clamp (11). Tissue-specific IR can also be modeled based on glucose and insulin responses during an oral glucose tolerance test (OGTT), which has been validated against the clamp technique (10, 12).

These tissue-specific IR phenotypes have previously been linked to distinct metabolic profiles, representing different etiologies toward T2DM and CVD (11, 13–15). More specifically, greater disturbances in circulating lipidome (13) and metabolome profiles (14) have been found in individuals with more pronounced LIR as compared to individuals with more pronounced MIR. Additionally, in individuals with LIR, abdominal subcutaneous adipose tissue (scAT) has been characterized by higher expression of genes related to extracellular modeling, whilst MIR has been associated with higher expression of genes related to inflammation in scAT, as well as higher levels of circulating plasma markers of systemic low-grade inflammation (16).

Recent findings indicate that these distinct metabolic phenotypes may respond differently to dietary macronutrient manipulation with regard to outcomes of glucose homeostasis, ectopic fat deposition, and tissue-specific lipid metabolism amongst others (15, 17). Indeed, a post-hoc analysis of the CORDIOPREV-DIAB study has indicated that a low-fat, high-complex carbohydrate diet may be particularly beneficial with respect to improvement in glucose metabolism for individuals with predominant

LIR, while individuals with predominant MIR seem to benefit more from a Mediterranean diet high in monounsaturated fatty acids (MUFA) (18). Therefore, further characterization of these IR phenotypes as well as studying these metabolic phenotypes in relation to dietary intervention outcomes may be a promising strategy to develop more personalized dietary interventions. In addition, improvement of glycemic control by more personalized dietary interventions may enhance mood, self-control, and cognitive function (1, 19–21). Such short-term benefits may in turn increase adherence to a healthy diet.

Importantly, prospective randomized controlled trials with a pre-specified hypothesis on differential metabolic responses to diets based on (metabolic) phenotype are largely lacking in the emerging field of precision nutrition. The PERSonalized glucose Optimization through Nutritional intervention (PERSON) study was designed to investigate the effects of an optimal compared to a suboptimal dietary intervention according to tissue-specific IR phenotype on glucose metabolism and other metabolic health outcomes. This two-center, 12-week dietary intervention study with a randomized, double-blind, parallel design, aims to enroll a total of 240 individuals with either LIR or MIR. Individuals are randomized to follow one of two diets that are hypothesized to target one of the two tissue-specific IR phenotypes.

Before and after the 12-week dietary intervention, individuals are extensively phenotyped both in laboratory settings and in daily life. The extensive phenotyping performed in this unique clinical trial allows for a comprehensive study of both the complex metabolic and lifestyle determinants of glucose homeostasis, as well as the dietary intervention effects on metabolic health and its metabolic underpinnings. In the present article, we describe the study design and measurements in detail, and present preliminary results of the screening population.

Methods

Study Design

The PERSON study is a two-center 12-week dietary intervention study with a randomized, double-blind, parallel design, carried out at Maastricht University Medical Center+ (MUMC+) and Wageningen University & Research (WUR), the Netherlands (Figure 1). The protocol was approved by the Medical Ethics Committee of MUMC+ (NL63768.068.17) and registered at ClinicalTrials.gov (identifier NCT03708419). The study is conducted according to the principles of the Declaration of Helsinki (revised version, 2013, Fortaleza, Brazil), and all subjects provide written informed consent before the start of the study.

The primary study outcome is the difference in change in disposition index, which is the product of insulin sensitivity and first-phase insulin secretion, between participants who received their hypothesized optimal or suboptimal diet. Secondary outcome parameters include whole-body and tissue-specific insulin sensitivity and glucose homeostasis, fasting and postprandial metabolic profile, vascular health, fecal microbiota composition and functionality, body fat distribution, ectopic fat accumulation, adipose tissue morphology and gene expression, skeletal muscle protein and gene expression, fasting immune metabolism, cognitive performance, and perceived well-being.

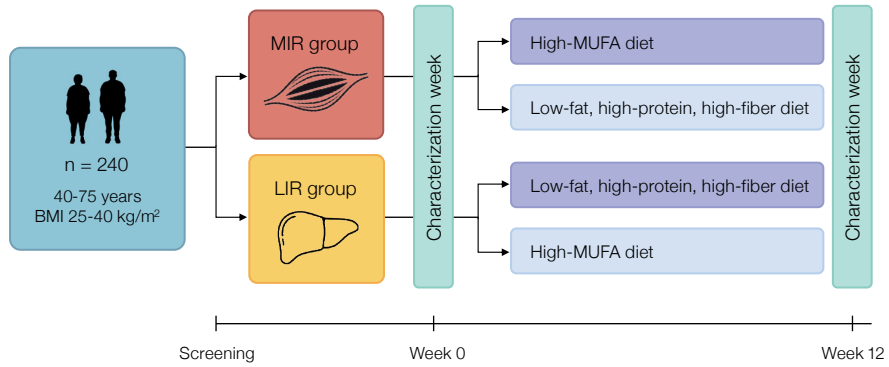

Figure 1. Study design of the PERSON study. Tissue-specific insulin resistance (MIR, muscle insulin resistance; LIR, liver insulin resistance) is assessed at screening using a 7-point oral glucose tolerance test and eligible participants with MIR or LIR are randomized to follow either their hypothesized optimal (dark purple) or suboptimal (light purple) diet for 12 weeks. Before and after the intervention, participants are extensively phenotyped during a "characterization week" in a controlled laboratory setting as well as in daily life. BMI, body mass index; MUFA, monounsaturated fatty acid.

Study Participants

From May 2018 onwards, subjects have been recruited via a volunteer database, flyers, and advertisements in local and online media. Inclusion criteria are age 40–75 years, body mass index (BMI) 25–40 kg/m^2, body weight stability for at least 3 months (no weight gain or loss >3 kg), and tissue-specific IR, characterized as predominant LIR or MIR, as assessed by a 7-point OGTT (see "Screening"). Exclusion criteria include among others pre-diagnosis of T2DM, diseases or use of medication that affect glucose and/or lipid metabolism, major gastrointestinal diseases, history of major abdominal surgery, uncontrolled hypertension, smoking, alcohol consumption >14 units/wk, and >4 h/wk moderate-to-vigorous physical activity (see Supplementary Table 1 for the extensive list of exclusion criteria).

Screening

Eligibility is assessed during a screening visit. Subjects are asked to refrain from alcohol and vigorous physical activity 24 h prior to the visit and arrive in the morning after a >10 h overnight fast. Body weight and height are measured in duplicate without shoes and heavy clothing to the nearest 0.1 kg and 0.1 cm, respectively. Waist and hip circumference are measured in duplicate to the nearest 0.1 cm using a non-flexible measuring tape. Blood pressure is measured in triplicate on the non-dominant arm with an automated sphygmomanometer after a 5-min rest with the subject in a supine position. The first measurement is used to acclimatize the subject to the measurements, and therefore omitted from the data.

Tissue-specific insulin resistance is assessed based on the glucose and insulin responses during a 7-point OGTT. Subjects ingest 200 ml of a ready-to-use 75 g glucose solution (Novolab) within 5 min, and blood samples are collected from the antecubital vein via an intravenous cannula under fasting conditions (t = 0 min) and after ingestion of the glucose drink (t = 15, 30, 45, 60, 90, and 120 min) for determination of plasma glucose and insulin concentrations. Hepatic IR and muscle insulin sensitivity are estimated using the calculations of Abdul-Ghani and colleagues (10). We have recently optimized the MISI calculator using the cubic spline method (12). The hepatic IR index (HIRI) and muscle insulin sensitivity index (MISI) are calculated according to the following formulas:

$$\text{HIRI} = \text{glucose 0-30 [}\textit{AUC in mmol/L} * \textit{h}\text{]} \times \text{insulin 0-30 [}\textit{AUC in pmol/L} * \textit{h}\text{]}$$

$$\text{MISI} = \frac{\text{dGlucose / dt}}{\text{insulin [}\textit{mean during OGTT in pmol/L}\text{]}}$$

In the formula for MISI, dG/dt is the rate of decay of plasma glucose concentration (mmol/L) during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir (10).

In the formula for MISI, dG/dt is the rate of decay of plasma glucose concentration (mmol/L) during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir (10).

Glucose curves that are flagged by the calculator, because MISI calculation is not possible or possibly not biologically meaningful due to either a peak at 120 min, a “flat” curve, or non-negligible rebound (12), are visually inspected for classification of MIR and LIR. Both indices were developed and validated against gold standard measurements of tissue-specific IR by a hyperinsulinemic-euglycemic clamp (10, 12). To obtain study groups that are predominant LIR or MIR, subjects are classified as “No MIR/LIR,” “MIR,” “LIR,” or “combined MIR/LIR,” using tertile cutoffs for MISI and HIRI. The lowest tertile of MISI represents individuals with MIR, while the highest tertile of HIRI represents individuals with LIR. The cutoffs for these tertiles are based on values of a selected study population of The Maastricht Study (DMS) (22), which resembles the target population of the PERSON study. Since the prevalence of LIR seems lower in the PERSON study as compared to DMS after inclusion of n = 163 individuals, the median HIRI value in the PERSON study population will be used for classification of individuals that will be recruited for the remainder of the study.

From the OGTT, incremental area under the curve (iAUC) is calculated for both glucose and insulin using GraphPad Prism software (version 5.04). Only values above the fasting value are included in the iAUC. The homeostasis model assessment of insulin resistance (HOMA-IR) is calculated as (fasting glucose [mmol/L] × fasting insulin [mU/L])/22.5 (23). HOMA of β-cell function (HOMA- β) is calculated as (20 × fasting insulin [mU/L])/(fasting glucose [mmol/L] – 3.5). Matsuda index is defined as: [10,000 ÷ square root of [fasting plasma glucose (mmol/l) × fasting insulin (pmol/l)] × [mean glucose (mmol/l) x mean insulin (pmol/l)]], using glucose and insulin values of time points 0, 30, 60, 90, and 120 min (24). Disposition index is calculated as: [Matsuda index * (AUC30 min insulin/AUC30 min glucose)], where AUC30 min is the area under the curve between baseline and 30 min of the OGTT for insulin (pmol/l) and glucose (mmol/l) as calculated using the trapezoidal method, respectively. Glucose status is defined according to WHO criteria (25): normal glucose tolerance (NGT), fasting glucose <5.6 mmol/L and 120-min glucose <7.8 mmol/L; impaired fasting glucose (IFG), fasting glucose 5.6 – 6.9 mmol/L and 120-min glucose <7.8 mmol/L; impaired glucose tolerance (IGT), fasting glucose <5.6 mmol/L and 120-min glucose 7.8 – 11.0 mmol/L; combined IFG/IGT, fasting glucose 5.6 – 6.9 mmol/L and 120-min glucose 7.8-11.0 mmol/L; T2DM, fasting glucose ≥7.0 mmol/L and/or 120-min glucose ≥11.1 mmol/L.

Hb and the parameters of hepatic and renal function alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine are determined in fasting blood samples by the hospital laboratories of MUMC+ and Ziekenhuis Gelderse Vallei, Ede, the Netherlands. Habitual dietary intake is estimated by a validated 163-item semiquantitative food frequency questionnaire (FFQ) (26). Dietary misreporting is evaluated by Goldberg’s method, using the ratio of daily energy intake (EI) to estimated basal metabolic rate (BMR) (27, 28). Energy under- (EI/BMR < 0.87) and overreporters (EI/BMR > 2.75) are excluded from data analyses. Data on demographics, medical history, family history of DM (≥1 first-degree relative with DM), medication use and lifestyle are collected by questionnaire. Education level is categorized into low (no education, primary education, lower or preparatory vocational education, lower general secondary education), medium (intermediate vocational education, higher general senior secondary education or pre-university secondary education) and high (higher vocational education, university). Perceived chronic

stress is assessed with the Long-term Difficulties Inventory (29) and mental well-being with the RAND 36-Item Short Form Health Survey (RAND-36) (30) and the Social Production Function Instrument for the Level of Well-being (31).

Randomization Procedure

Eligible subjects are randomly allocated to either their hypothesized optimal or suboptimal diet by an independent analyst using center-specific minimization (32, 33) with randomization factors of 1.0 for the LIR/MIR phenotype, and 0.8 for age and sex, and a base probability of 0.7 by means of biased-coin (34). Both researchers and participants are blinded to the participants' metabolic phenotype, and thus blinded to whether participants are allocated to their hypothesized optimal or suboptimal diet. Participants start the study within 3 months of the screening visit.

Dietary Intervention

The hypothesized optimal diet for MIR is a moderate-fat diet high in MUFA (HMUFA) with a targeted macronutrient composition of 38% of energy from fat (20% MUFA, 8% PUFA, 8% SFA), 48% of energy from carbohydrates (CHO) (30% polysaccharides; 3 g/MJ fiber), and 14% of energy from protein (Table 1). The hypothesized optimal diet for LIR is low in fat, and high in protein (LFHP) and fiber. Energy from CHO is similar between diets. The targeted macronutrient composition of the LFHP diet is composed of 28% of energy from fat (10% MUFA, 8% PUFA, 8% SFA), 48% of energy from CHO (30% polysaccharides; >4 g/MJ fiber), and 24% of energy from protein (Table 1).

The dietary intervention strategy is based on intensive dietary counseling and provision of key products. Before the start of the intervention, a short dietary history is performed to assess the participants' dietary habits and preferences. This information is used to individualize the dietary plan and counseling accordingly. Participants are assigned to one of eight energy groups ranging from 6 to 13 MJ/d according to their estimated individual energy requirement, which is calculated by averaging self-reported energy intake from the FFQ with the product of the predicted BMR, as calculated with Schofield equations (35), and self-reported physical activity level.

At the start of the intervention period, participants receive verbal and written instructions on their dietary plan, which lists both types and quantities of foods that they are required to consume daily or weekly in order to meet the targeted nutrient composition of the assigned diet. The instructions include guidance on what types of foods to choose and avoid within all food groups (e.g., what grain products are [not] allowed; what type and cut of meat or poultry is [not] allowed). Intake of so-called free-food items (e.g., from caloric sweeteners, sweets, sweet spreads, cookies, fruit juice, sugar-sweetened and/or alcoholic beverages) is restricted to 5–10% of energy intake in both diets. The individual dietary plans include a number of "points" per day that have to be "spent" on such foods.

Table 1. Targeted nutrient composition of the HMUFA and LFHP diet

	HMUFA	LFHP
Fat (en%)	38	28
Monounsaturated fat	20	10
Polyunsaturated fat	8	8
Saturated fat	8	8
Protein (en%)	14	24
Animal-based, % of total protein	45	60
Plant-based, % of total protein	55	40
Carbohydrates (en%)	42	42
Mono- and disaccharides	12	12
Polysaccharides	30	30
Fiber, g/MJ	3	>4
Alcohol	<3	<3

en%, energy percentage of total energy intake; MJ, megajoule

Key products that largely distinguish the two diets with regards to macronutrient composition are provided in pre-measured amounts. For the HMUFA diet, key products include olive oil, olives, olive tapenade, and low-fat margarine with olive oil. Key products for the LFHP diet include low-fat yogurt and quark, reduced-fat cheese, very low-fat spread, pumpkin seeds, baking margarine with olive oil, and a dietary fiber supplement (2 g β-glucan per 6 g, PromOat®, DSM Nutritional Products, Basel, Switzerland) providing 6–12 g of additional fiber per day. Participants are instructed to finish a certain amount of every provided product each day. Apart from the fiber supplement, all products are commercially available. Alcohol consumption is restricted to ≤ 1 glass/day, in agreement with the current Dutch dietary guidelines (36).

Throughout the intervention period, participants visit the research facilities every week for a 15- to 30-min individual dietary counseling session with a dietitian or research nutritionist to monitor diet adherence, body weight, and adverse events using a semi-structured interview. These sessions are supported by advice via e-mail or telephone if needed. To be able to assess the effects of the dietary intervention on metabolic health parameters, independent of changes in body weight, we aim to keep participants on a stable body weight throughout the study. In case of weight loss or gain, participants are reassigned to a higher or lower energy group to prevent further weight change. To promote overall diet adherence, participants are allowed to deviate from their dietary plan on three individual days throughout week 2–10 of the intervention period. Participants are asked to keep a food record (FR) on these days.

During the COVID-19 restrictions, the weekly on-site visits are replaced by telephone or video-call consultations, key products are home-delivered by courier, and participants weigh themselves at home.

Dietary compliance is assessed by three unannounced 1-day FR with the mobile app "Traqq" (37) on 2 non-consecutive weekdays and 1 weekend day. Participants are provided with written and face-to-face instructions on how to record dietary intake. Participants that do not have a smartphone complete the FRs on paper, which are later entered into the app by the researcher.

Measurements

In the week before start of the intervention and in the last week of the 12-week intervention, participants are extensively phenotyped during a "characterization week" (Figure 2). This week includes three or four (depending on study center and participation in additional subgroup measurements) clinical test days and three at-home days. Participants wear a continuous glucose monitor (CGM) and activity monitor throughout the characterization week. During the clinical test days, participants undergo extensive laboratory testing, which includes challenge tests, body composition analysis, vascular measurements, tissue biopsies, a cognitive test, and questionnaires. During the at-home days, participants record dietary intake and feelings of well-being, consume various standardized meals, and collect feces and urine. An overview of all measurements can be found in Figures 2, 3 and are described in more detail below.

On the clinical test days, participants are instructed to travel to the facility by car or public transport. The day prior to and during the characterization weeks, participants are requested to refrain from alcohol and vigorous physical activity. In the week before the baseline characterization week, participants record their dietary intake for three random days (2 week days and 1 weekend day) using the mobile app "Traqq" (37)

Laboratory Challenge Tests

A 7-point OGTT is performed according to the same procedures used at screening (see "Screening") (Figures 3, 4). Participants consume a standardized low-fat macaroni meal (30% of energy intake [en%] fat, 49 en% CHO, 21 en% protein; 1,560–2,460 kJ, depending on energy group) the evening before the OGTT, after which they remain fasted until the OGTT. The macaroni meal is prepared in the university kitchen. A fasting blood sample is drawn for determination of glycated hemoglobin (HbA1c) by the hospital laboratories of MUMC+ and Ziekenhuis Gelderse Vallei, Ede, the Netherlands.

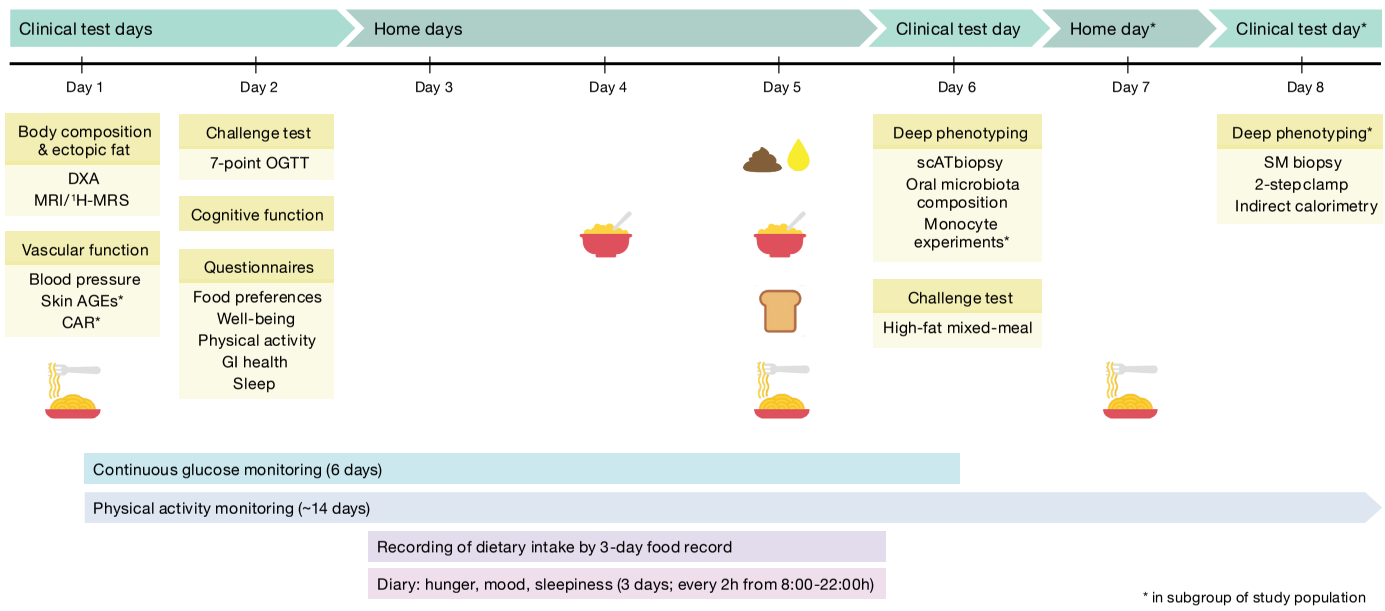

Figure 2. Graphical overview of the pre- and post-intervention characterization week. The characterization week contains multiple clinical test days, during which participants are extensively phenotyped. In addition, blood glucose and physical activity are continuously monitored. At home, participants record their dietary intake and feelings of well-being, collect a fecal sample and 24-h urine, and consume a standardized breakfast on day 4, and on day 5, participants have a full day of standardized meals and snacks, including the standardized breakfast. In a subgroup of the study population, additional measurements are performed. DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; 1H-MRS, proton magnetic resonance spectroscopy; AGEs, advanced glycation endproducts; CAR, carotid artery reactivity; OGTT, oral glucose tolerance test; GI, gastrointestinal; scAT, subcutaneous adipose tissue; SM, skeletal muscle.

	Method	Main parameters	Study time point			
			SCR	CW1	DIW	CW2
Laboratory challenge tests	Oral glucose tolerance test	Glucose homeostasis parameters	•	•		•
	High-fat mixed-meal test	Fasting and postprandial metabolic profile		•		•
Body composition, fat distribution and ectopic fat	Digital weighing scale	Body weight	•	•	•	•
	Measuring tape	Waist and hip circumference	•	•		•
	Magnetic resonance imaging (MRI)	Abdominal subcutaneous adipose tissue, visceral adipose tissue, intrahepatic lipid content*, muscle fat infiltration*		•		•
	Proton magnetic resonance spectroscopy (^{1}H-MRS)*	Intrahepatic lipid content		•		•
	Dual-energy X-ray absorptiometry (DXA)	Whole-body and regional fat mass, lean body mass, body fat percentage, bone mineral density		•		•
Cardiovascular measurements	Automated sphygmomanometer	Systolic and diastolic blood pressure	•	•		•
	Carotid artery diameter responses to a cold pressor test*	Carotid artery reactivity		•		•
	AGE-reader*	Skin accumulation of advanced glycation end-products		•		•
Dietary intake	Food frequency questionnaire	Habitual dietary intake	•			
	3x 1-day food record	Actual dietary intake		•	•	•
Microbiota composition and functionality	Feces collection	Fecal microbiota composition, fecal SCFA		•		•
	Bristol stool chart	Self-reported stool consistency		•		•
	Rome III criteria	Self-reported gastrointestinal health		•		•
	Oral sample collection	Oral microbiota composition		•		•
Deep laboratory phenotyping	Abdominal subcutaneous adipose tissue biopsy	Gene and protein expression, adipocyte size, immune cell composition*		•		•
	Skeletal muscle biopsy in *m. vastus lateralis**	Gene and protein expression		•		•
	2-step hyperinsulinemic-euglycemic clamp*	Whole-body and tissue-specific insulin resistance, substrate oxidation		•		•
	Ex vivo monocyte experiments*	Fasting immune metabolism	•	•		•
	24-hour urine collection	Urinary metabolites		•		•
Measurement in daily life	Continuous glucose monitoring (6 days)	Glycemic variability, glycemic response to standardized meals		•		•
	Physical activity monitoring (~14 days)	Sedentary and physical activity parameters		•		•
	Likert scales (every 2 hrs for 3 days)	Self-reported mood, hunger, and sleepiness		•		•
Cognitive performance	Cambridge Neuropsychological Test Automated Battery (CANTAB)	Executive function, memory, attention & psychomotor speed		•		•
Questionnaires	General questionnaire	Demographics, medical history, medication use, lifestyle	•			
	Long-term Difficulties Inventory	Perceived chronic stress	•			
	Social Production Function Instrument for the Level of Well-being	Mental well-being	•			
	RAND 36-item Health Survey	Mental well-being	•	•		•
	Perceived Stress Scale-10, Chalder Fatigue scale	Mental well-being		•		•
	PSQI, Munich ChronoType, Epworth sleepiness scale	Sleep quality and characteristics		•		•
	Baecke questionnaire, AQuAA	Self-reported physical (in)activity		•		•

Figure 3. Overview of all measurements performed within the PERSON study.

*Performed in a subgroup of the study population. SCR, screening visit; CW, characterization week; DIW, dietary intervention week.

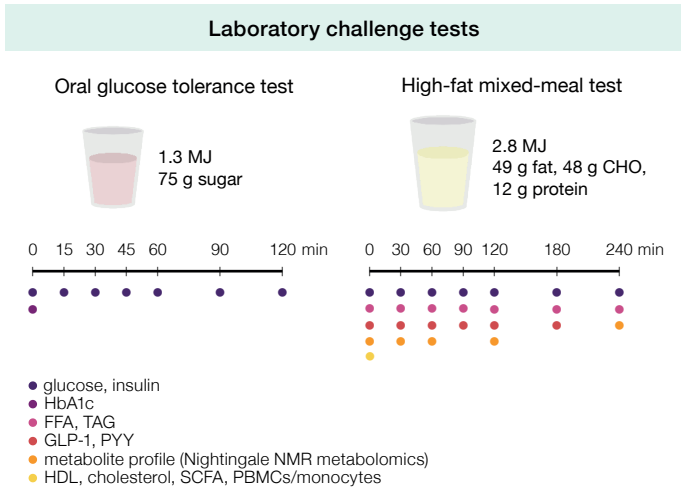

Figure 4. Graphical overview of the oral glucose tolerance test and the high-fat mixed-meal (HFMM) test that are performed during the pre- and post-intervention characterization week. Participants are instructed to drink the glucose drink or HFMM within 5 min, and fasting and postprandial blood samples are drawn at the indicated timepoints for determination of the indicated metabolites. CHO, carbohydrates; HbA1c, hemoglobin A1c; FFA, free fatty acids; TAG, triglycerides; GLP-1, glucagon-like peptide 1; PYY, peptide YY; NMR, nuclear magnetic resonance; HDL, high-density lipoprotein; SCFA, short-chain fatty acids; PBMCs, peripheral blood mononuclear cells.

On a separate clinical test day, at least 4 days after the OGTT, a high-fat mixed-meal (HFMM) challenge test is performed after a 12-h overnight fast (Figures 3, 4). Participants again consume the standardized low-fat macaroni meal the evening before the test. The liquid HFMM (350 g containing 2.8 MJ, 49 g [64 en%] fat, 48 g [29 en%] carbohydrate, 12 g [7 en%] protein) is prepared in the university kitchen using whipped cream ice cream, whipped cream, full-fat milk, and sugar (Supplementary Table 2). An intravenous cannula is inserted in the antecubital vein for blood sampling. At least 30 min following insertion of the catheter, a fasting blood sample is drawn (t = 0 min). Subsequently, participants are asked to consume the liquid HFMM within 5 min and postprandial blood samples are drawn at t = 30, 60, 90, 120, 180, and 240 min for determination of glucose, insulin, free fatty acids (FFA), triacylglycerol (TAG), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) (Figure 4). Total cholesterol and HDL cholesterol are determined in fasting serum. Extensive plasma metabolite profiling is performed in samples from T = 0, 30, 60, 120, and 240 min by high-throughput nuclear magnetic resonance (NMR) metabolomics (Nightingale Health Ltd., Helsinki, Finland) (38). Buffy coat is collected from fasting blood for later DNA isolation and genotyping. At each blood drawing, participants rate their hunger, fullness, satiety, thirst, and desire to eat on a 100-mm Visual Analog Scale (VAS), anchored at the extremes “not at all” to “extremely.”

Cardiovascular Markers

Blood pressure is assessed according to the same procedures used at screening. In a subgroup of participants, vascular function is assessed by measuring carotid artery reactivity (CAR) to a cold pressor test (CPT) (39). After 10 min of rest in supine position, the participant’s left hand is submerged in a bucket of icy water (≤ 4°C) for 3 min. The diameter of the left common carotid artery is monitored during a 1-min baseline assessment and continuously during the 3-min CPT using ultrasound (Terason uSmart 3300, Burlington, MA, USA). Wall-tracking and edge-detecting software is used to calculate the diameter after completion of the test. To confirm sympathetic stimulation, blood pressure is measured after the supine rest, 1-min and 2- min after the start of the CPT, and directly after completion of the CPT (Omron M6 Comfort, Omron healthcare Co., Ltd., Kyoto, Japan).

In a subgroup, skin accumulation of advanced glycation end-products (AGE) is measured by skin autofluorescence (AF) using the automated AGE reader (DiagnOptics Technologies B.V., Groningen, the Netherlands). Skin AF is measured at three slightly different places on the volar side of the dominant arm, avoiding impurities of the skin such as scars and birthmarks. Participants are instructed to not apply any creams, lotions, or sunscreen on their arms on the day of the measurement.

Body Composition, Fat Distribution, and Ectopic Fat Deposition

Body weight is measured in underwear, and waist and hip circumference are measured according to the procedures described earlier (see “Screening”). Whole-body and regional fat mass, fat percentage, lean body mass, and bone mineral density are assessed using dual-energy X-ray absorptiometry (DXA), while participants are fasted for ≥2 h (MUMC+, Discovery A, Hologic; WUR, Lunar Prodigy, GE Healthcare) (Figure 3).

At MUMC+, a whole-body scan is made after a ≥2 h fast with a 3T magnetic resonance imaging (MRI) scanner (3T MAGNETOM Prisma fit, Siemens Healthcare), using a radiofrequency transmit/receive body coil at Scannexus, Maastricht, the Netherlands. Analyses are performed using a computational modeling method [AMRA Medical AB, Linköping, Sweden (40)] for quantification of abdominal subcutaneous adipose tissue (ASAT), visceral adipose tissue (VAT), thigh muscle volume, intrahepatic lipid content (IHL), and muscle fat infiltration (MFI) in the anterior thighs (Figure 3).

At WUR, IHL and abdominal fat distribution are assessed with proton magnetic resonance spectroscopy (^{1}H-MRS) and MRI, respectively, on a 3T whole-body scanner (Siemens, Munich, Germany; Philips Healthcare, Best, the Netherlands from

November 2020 onwards). MRI measurements are performed after a ≥2 h fast at hospital Gelderse Vallei, Ede, the Netherlands. Spectra for determination of IHL are obtained from a 30 × 30 × 20 mm voxel placed in the right lobe of the liver, avoiding blood vessels and bile ducts. Participants are instructed to hold their breath when spectra are acquired to reduce respiratory motion artifacts. Spectra are post-processed and analyzed using the AMARES algorithm in jMRUI software. Abdominal fat distribution is evaluated as subcutaneous (ASAT) and visceral adipose tissue (VAT) areas in the abdomen, which are quantified in singles-slice axial T1-weighted spin echo transverse images at the inter-vertebral space L3-L4 using the semi-automatic software program HippoFatTM (41).

Microbiota Composition and Functionality

During one of the at-home days in the characterization week, participants collect fecal samples (Figures 2, 3). The samples are stored in the participants' home freezer for maximal 72 h before the visit to the research facilities. Participants rate stool consistency of the sample using the Bristol stool scale (42). Fecal microbiota composition is determined by 16S rRNA sequencing as described elsewhere (43).

During the HFMM challenge test, fasting and postprandial blood samples are collected for determination of plasma concentrations of GLP-1 and PYY (Figure 3). Fecal concentrations and fasting plasma levels of gut microbiota-derived short-chain fatty acids (SCFA) acetate, propionate and butyrate are determined using optimized LC-MS protocols (44).

Data on self-reported gastrointestinal health are collected by a questionnaire based on the Rome III criteria (45). The questionnaire includes questions on presence of gastrointestinal complaints (i.e., abdominal pain, obstipation, bloating), defecation frequency, and stool consistency (Figure 3).

In addition, oral samples are collected for microbiological and metabolite analyses. Participants are asked to rinse the oral cavity thoroughly for 30 s with 10 ml of sterile 0.9% saline and expectorate the rinse in a tube. The tube is kept on ice, vortexed and the rinse is aliquoted, snap-frozen in liquid nitrogen and stored at −80 °C for later analysis. Participants are instructed to refrain from oral hygiene in the morning of the sampling day. The composition of the oral microbiome is determined by 16S rRNA sequencing (46).

Deep Laboratory Phenotyping

Abdominal Subcutaneous Adipose Tissue Biopsy

On the morning of the HFMM, an abdominal SAT biopsy is collected 6-10 cm lateral from the umbilicus under local anesthesia (1% lidocaine) by needle biopsy. The samples are washed with saline to remove blood clots. A portion of tissue is fixed overnight at 4°C in 4% paraformaldehyde and embedded in paraffin for histological sections to determine adipocyte morphology. In a subgroup of participants, at baseline only, ~0.7 g of fresh AT is used for fluorescence activated cell sorting (FACS) analysis. In short, the stromal vascular fraction is isolated from the AT and stained with a cocktail of antibodies for flow cytometry for identification of immune cells (47). The remaining tissue is snap-frozen in liquid nitrogen and stored at −80 °C for later analyses of targeted gene and protein expression.

Skeletal Muscle Biopsy

In a random subgroup of participants at MUMC+ (n = 60 in total; n = 15 per intervention group), a skeletal muscle (SM) biopsy is collected and a two-step hyperinsulinemic-euglycemic clamp is performed on a separate clinical test day at the end of the characterization week (Figure 3). The skeletal muscle biopsy is taken from the *m. vastus lateralis* under local anesthesia using the Bergström biopsy needle method (48). After removal of blood and fat tissue, a portion of the biopsy is snap-frozen in melting isopentane and stored at −80 °C for biochemical analyses. The remaining tissue is snap-frozen in liquid nitrogen and stored at −80 °C for later gene and protein expression analyses.

Two-Step Hyperinsulinemic-Euglycemic Clamp

After the SM biopsy, whole-body and tissue-specific insulin sensitivity are assessed by the gold standard two-step hyperinsulinemic-euglycemic clamp (49). At t = −120 min, primed D-[6.6-$^2H_2$] glucose tracer is started and infused continuously at 0.04 mg/kg/min, to allow calculations of rates of endogenous glucose production (EGP), glucose appearance (Ra), and glucose disposal at basal conditions. At t = 0, a low primed constant co-infusion of insulin at 10 mU/m^2/min is started for 3 h for determination of hepatic insulin sensitivity. At t = 180 min, the primed constant infusion of insulin is increased to 40 mU/m^2/min for 2.5 h to inhibit EGP and measure muscle insulin sensitivity. Arterialized blood is frequently sampled from the superficial dorsal hand vein during the insulin infusion to measure glucose concentrations, which are maintained at ~5.0 mmol/L by a co-infusion of 20% glucose at variable rate (GIR). Substrate utilization is measured for 30 min during the basal, low insulin, and high insulin infusion using indirect calorimetry by ventilated hood (Omnical, Maastricht Instruments, Maastricht). Resting metabolic rate (RMR), fat and carbohydrate oxidation are calculated according to the equations of Weir and Frayn (50, 51). The clamp is performed after an overnight (≥12 h) fast and participants consume the standardized macaroni meal the evening before the clamp.

Fasting Immune Metabolism

At WUR only, circulating peripheral blood mononuclear cells (PBMCs) are isolated from fasted blood samples collected at the HFMM test (Figure 3). In addition, in a

random subgroup (*n* ~ 200), PBMCs are also isolated from fasted blood samples collected at screening. PBMCs are isolated by density gradient isolation using CPT tubes (BD vacutainer, cat. no. 362753). Monocytes are subsequently obtained by MACS (magnetic activated cell sorting) positive selection using CD14 MicroBeads (Miltenyi Biotec, cat no. 130-050-201). Part of the monocytes are exposed overnight (24 h) to the inflammatory stimuli lipopolysaccharide (LPS) (10 ng/mL, sigma, cat. no L6529) and P3C (10 ug/mL, EMC collections, cat. no. L2000). Functional properties of monocytes are determined after treatment by measuring the release of cytokines including IL-6, IL-1b and CXCL8 (R&D DuoSet ELISA, cat. no. DY206; DY201; DY208). The metabolic potential of monocytes is measured in real-time experiments (inflammatory cell activation test and glycolytic stress test) using the Seahorse apparatus (Agilent Technologies) in screening samples only.

24-h Urine Collection

Participants collect 24-h urine in 2-3 liter containers containing 5 ml/L of 4 mM hydrochloric acid (HCl). Urine collection starts after the first voiding on the morning of the home-day with only standardized meals and finishes 24 h later on the morning of the HFMM. Participants are asked to store the containers in a cool place, preferably a refrigerator, and bring the containers to the facilities on the day of the HFMM. The urine of each participant is mixed, weighted, aliquoted, and stored at –80 °C for later analysis.

Measurements in Daily Life and At-Home Days

Continuous Glucose Monitoring

At the start of the characterization week, a CGM (Medtronic iPro2 with Enlite sensor) is placed lateral to the umbilicus for 6 days of continuous interstitial fluid glucose measurements (Figure 2). The CGM data are calibrated according to the manufacturer's instructions with four daily capillary glucose self-measurements using a blood glucose meter (Contour XT, Ascensia Diabetes Care).

To assess glycemic variability and glycemic responses to standardized meals, on one of the home-days, participants consume a standardized breakfast, and on another home-day, participants have a full day of standardized meals and snacks, including the standardized breakfast (Figure 2; Supplementary Tables 3, 4). Participants are instructed to consume the meals according to standardized instructions including time frames, to fast for 2 h after the breakfast, and to only drink water alongside the standardized meals.

Physical Activity Assessment

Physical activity is continuously monitored for ~14 days during both the characterization weeks and ~7 days in free-living conditions–either starting with the characterization week at baseline, or ending with the characterization week in week 12 (Figure 2)–using a triaxial accelerometer (activPAL3™ micro, PAL Technologies Ltd., Glasgow, Scotland, UK) attached to the middle of the right thigh. Participants keep a diary to record the times they go to sleep and wake up while wearing the monitor. Sedentary and physical activity parameters are quantified with a modified version of the script of Winkler et al. (52), using the sleeping and waking times as input.

Dietary Intake, Hunger, Mood, and Sleepiness

During the 3 at-home days, participants record their dietary intake including consumption of the standardized meals using the mobile app "Traqq" (37). In addition, participants are asked to report on hunger, mood, and sleepiness every 2 h from 8:00 to 22:00 h (Figure 2). Hunger is rated on an 11-point Likert scale ranging from "not hungry" to "very hungry." Self-reported mood is assessed with an adapted form of the Multidimensional Mood Questionnaire (MDMQ) (53). The 7-point scale consists of six bipolar items to assess the three basic dimensions of mood valence, calmness, and energetic arousal: tired/awake, satisfied/dissatisfied, agitated/calm, full of energy/without energy, unwell/well, and relaxed/tense. Sleepiness is rated on the 9-point Karolinska Sleepiness Scale, with labels ranging from "extremely alert" to "very sleepy, great effort keeping awake, fighting sleep" (54, 55).

Cognitive Performance

Cognitive performance is assessed in the domains of executive function, memory, and attention & psychomotor speed using the Cambridge Neuropsychological Test Automated Battery (CANTAB) (56). Executive function is evaluated with the multitasking test and spatial span test; memory with the delayed matching to sample test and paired associates learning test; and attention and psychomotor speed is assessed with the motor screening task and reaction time task. Each test is preceded by standardized instructions and a practice round for familiarization. Participants consume a standardized brunch containing of bread with cheese and/or ham and a caffeine-free drink before performing the test battery.

Self-Reported Food Preferences, Eating Rate, Sleep, Well-Being, and Physical (In)-activity

After the CANTAB, participants complete the computer-based Macronutrient and Taste Preference Ranking Task (MTPRT) for assessment of food preferences (57). The task assesses liking and ranking for 32 food products that are categorized as high in carbohydrates, high in fat, high in protein, or low-calorie, as well as either sweet or savory. Furthermore, participants rate their eating rate in comparison to others on a 5-point Likert scale with labels ranging from "very slow" to "very fast" (Figure 3).

In addition, during one of the clinical test days, participants provide information on general well-being, sleep characteristics, and physical (in)activity by questionnaire (Figure 3). Mental well-being is assessed using the RAND-36 (30) and perceived stress is measured with the 10-item Perceived Stress Scale (PSS-10) (58). Physical and mental fatigue are assessed using the 14-item Chalder fatigue scale (59). Sleep quality is assessed with the 10-item Pittsburgh Sleep Quality Index (60) and sleep duration and chronotype are derived from the Munich ChronoType Questionnaire (61). Daytime sleepiness is assessed with the 8-item Epworth Sleepiness scale (62) (Figure 3).

Self-reported habitual physical activity and sedentary behavior are assessed using the Baecke questionnaire (63) and the Activity Questionnaire for Adults and Adolescents (AQuAA) subscale "sedentary leisure time activities" (64), respectively. In addition, physical activity self-efficacy is measured with 5 items from a health specific self-efficacy scale (65) and physical inactivity temptations are assessed using the 5-item subscale "competing demands" from the Temptation to not Exercise Scale (66), extended with the item "How tempted are you not to exercise and be sedentary while being on a business trip?".

Biochemical Analyses of Blood Samples and Biobanking

A wide range of biological samples are collected in the present study, including blood plasma and serum, SAT, SM tissue, feces, urine, saliva, and PBMCs. EDTA (Becton Dickinson, Eysins, Switzerland) tubes are centrifuged at 1,200 g, 4°C for 10 min and plasma is aliquoted subsequently. Serum tubes are left at room temperature for at least 30 min to allow clotting after sampling and centrifuged at 1,200 g, 20°C for 10 min before aliquoting of serum. All biological samples are snap-frozen in liquid nitrogen and stored at −80°C until analysis. Samples from both centers are analyzed at central laboratories. Plasma glucose, insulin, and FFA are measured on a Cobas Pentra C400 using ABX Pentra Glucose HK CP reagens (Horiba ABX Diagnostics, Montpellier, France), ELISA (Meso Scale Discovery, Gaithersburg, USA), and NEFA HR (2) reagens (2) (Wako chemicals, Neuss, Germany), respectively. Serum TAG, total cholesterol, and HDL cholesterol are measured on a Cobas Pentra C400 using ABX Pentra Triglycerides HK CP reagens, ABX Pentra Cholesterol CP reagens, and ABX Pentra HDL Direct, respectively. During the HFMM challenge test, fasting and postprandial blood samples are collected in EDTA tubes and aprotinin tubes containing dipeptidyl peptidase-IV inhibitor (Milipore Merck, Billerica, MA, USA) for determination of plasma GLP-1 and PYY, respectively. Total GLP-1 immunoreactivity is assessed using an antiserum that reacts equally with intact GLP-1 and the primary (N-terminally truncated) metabolite as previously described (67). PYY concentrations are determined with a commercially available radioimmunoassay for Human PYY (3-36) (Millipore Corporation, MA, USA).

Data Management

Data are collected on paper case report forms (CRF) and are entered in an electronic CRF designed for the study, using the web-based data capturing platform Caster EDC (68) that is compliant with good clinical practice (GCP) requirements. All relevant raw and processed data (e.g., from blood analyses, DXA scan) are also added to the eCRF in Castor EDC. Data entered in the eCRF are checked against the paper CRF by a study team member that did not enter the data. Data are collected and stored according to the FAIR (Findability, Accessibility, Interoperability, and Reusability) principles (69). A central data manager monitors data entry of both centers, performs data cleaning, and ensures that inaccurate or missing data are addressed.

Sample Size Calculation

Based on previous data, we expect a greater improvement in disposition index in participants receiving their hypothesized optimal diet compared to those receiving their hypothesized suboptimal diet (10). Data from the previously published DiOGenes study (18) as well as the CORDIOPREV-DIAB Study (18) were used to calculate an average standardized effect size from the difference in outcome values between the optimal and suboptimal diets in those studies. For DiOGenes, the low vs. high GI diets during the weight regain period were used and for CORDIOPREV the Mediterranean vs. low fat-high complex carbohydrate diets were used, in interaction with either MIR or LIR. With a power of 90%, two-sided alpha of 5% and a standardized effect size of 0.46, a total sample size of 202 was calculated using the statistical analysis software R. Taking into account a drop-out rate of 15%, 240 subjects will be included.

Statistical Analyses

In this paper, preliminary screening data from May 2018 to March 2020 are included. Baseline characteristics were compared between the four IR phenotypes (No MIR/LIR, MIR, LIR, combined MIR/LIR), using one-way ANOVA with Bonferroni *post-hoc* pairwise comparisons for numerical data (mean ± SD), and using Fisher's exact test for categorical data (%). Parameters of glucose homeostasis from the OGTT and dietary intake data from the FFQ were log-transformed due to non-normality, and differences between the IR phenotypes were tested using ANCOVA with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons. Statistical analyses were performed in SPSS (version 25.0). Differences in glucose and insulin responses following the OGTT between the IR phenotypes were tested using linear mixed-effects models (LMM) with Bonferroni *post-hoc* pairwise comparisons. The time courses of glucose and insulin were modeled with third-order (cubic) orthogonal polynomials. The effect of IR phenotype on all time terms and sex were included as fixed effects with participant random effects on all time terms. The adequacy of the higher order

polynomials was assessed with a likelihood-ratio test between nested models. The covariance matrix of the residuals was modeled as an unstructured matrix and model parameters were estimated using maximum likelihood estimation in all models. Estimated marginal means (EMM) with the degrees of freedom and corresponding *p*-values were estimated using Satterthwaite's method. All mixed-effects models were implemented using the "lmer" function of the lme4 package and EMMs were computed using the emmeans package in R (version 3.3.3, The R foundation for Statistical Computing, http://www.r-project.org/).

Results

Between May 2018 and March 2020, 632 individuals were enrolled, of whom 565 were fully screened for eligibility (Figure 5). In total, 40.2% of fully screened individuals were classified as No MIR/LIR, 21.4% as MIR, 10.8% as LIR, and 27.6% as combined MIR/LIR. Here, we present the characteristics of the study participants that have thus far been screened in the present ongoing clinical trial.

Baseline Characteristics

Baseline characteristics of all participants that completed screening are reported according to IR phenotype in Table 2. Mean age of the four groups (60 – 62 years) was comparable. The proportion of women in the total study population was 59% and was higher in the MIR group (69%) compared to the other groups, but only statistically significantly different from the combined MIR/LIR group. Individuals with combined MIR/LIR had higher BMI, waist circumference, waist-to-hip ratio, systolic and diastolic blood pressure, ALT levels, and use of antihypertensive medication compared to the No MIR/LIR, MIR, and LIR groups. Anthropometric and clinical characteristics were similar between the MIR and LIR group. IFG was most prevalent in the LIR group (11.5%), while IGT was most prevalent in the MIR group (18.2%) and the combined MIR/LIR group (16.7%). The prevalence of newly diagnosed T2DM was 6.6%, 8.3%, 0.0%, and 10.9% in the No MIR/LIR, MIR, LIR, and combined MIR/LIR group, respectively.

Glucose Homeostasis

By definition, both plasma glucose and insulin curves throughout the OGTT differed between the IR groups ($p < 0.001$ for both; Figure 6). Throughout the first 30 min of the OGTT, plasma glucose concentrations were higher in the LIR group compared to the MIR group (Figure 6A). Plasma insulin concentrations were higher in the LIR group compared to the MIR group at timepoints 15 – 60 min, whereas at 120 min, insulin was lower in LIR compared to MIR (Figure 6D). The iAUCs of both glucose and insulin

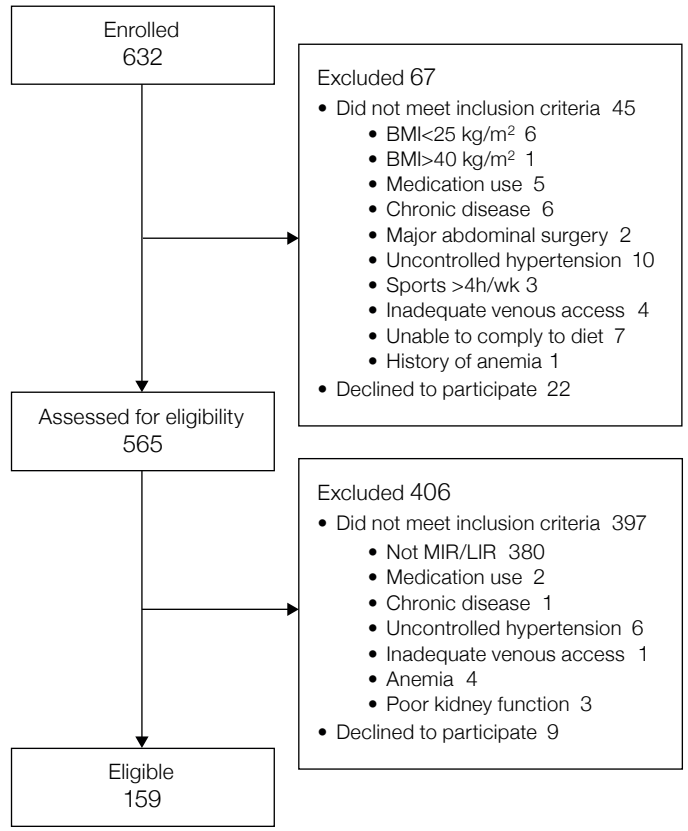

Figure 5. Flowchart of participant enrollment and eligibility from March 2018 to March 2020.

were lowest in the No MIR/LIR group, highest in the combined MIR/LIR group, and comparable between the MIR and LIR group (overall $p < 0.001$; Figures 6C,F), as were HOMA-IR (overall $p < 0.001$; Figure 7A) and HOMA-β ($p < 0.001$; Figure 7B). Similarly, Matsuda index was highest in the No MIR/LIR group, lowest in the combined MIR/LIR group, and comparable between the MIR and LIR group (overall $p < 0.001$; Figure 7C). Disposition index was higher in the LIR group compared to the other groups (overall $p = 0.002$; Figure 7D). Furthermore, by definition, MISI was lowest in the combined MIR/LIR and the MIR group (overall $p < 0.001$; Figure 7E) and HIRI was highest in the combined MIR/LIR and LIR group (overall $p < 0.001$; Figure 7F). All analyses were adjusted for sex. Values of these glucose homeostasis parameters derived from OGTT are reported in Supplementary Table 5.

Table 2. Characteristics of screened participants according to insulin resistance phenotype

	No MIR/LIR (n=227)	MIR (n=121)	LIR (n=61)	Combined MIR/LIR (n=156)	*P*-value
Age (years)	61 ± 9	60 ± 9	61 ± 8	62 ± 8	0.627
Women (%)	59.9	69.4	54.1	52.6†	0.031
Weight (kg)	86.6 ± 13.1	86.0 ± 11.2	86.4 ± 11.8	94.0 ± 14.7§†‡	<0.001
BMI (kg/m^2)	29.2 ± 3.3	29.8 ± 3.1	29.6 ± 3.2	32.2 ± 4.1§†‡	<0.001
Waist circumference (cm)	98.5 ± 10.6	100.4 ± 9.5	99.9 ± 9.5	106.3 ± 11.0§†‡	<0.001
Waist-to-hip ratio	0.91 ± 0.09	0.92 ± 0.08	0.94 ± 0.09	0.95 ± 0.09§†	<0.001
SBP (mmHg)	132 ± 17	132 ± 13	132 ± 16	137 ± 16§	0.015
DBP (mmHg)	80 ± 11	80 ± 10	80 ± 11	85 ± 10§†‡	<0.001
Hemoglobin (mmol/L)	8.8 ± 0.7	8.7 ± 0.7	8.8 ± 0.7	9.0 ± 0.8§†	0.003
Creatinine (µmol/L)	75.0 ± 14.1	73.8 ± 14.1	76.0 ± 16.2	78.4 ± 14.7	0.051
ALT (IU/L)	23 ± 10	27 ± 12	25 ± 9	31 ± 14§†‡	<0.001
AST (IU/L)	22 ± 6	22 ± 7	23 ± 6	25 ± 8§†	0.002
Use of statins (%)	9.7	6.6	11.5	13.5	0.293
Use of antihypertensives (%)	17.2	15.7	14.8	28.2	0.022
Family history of DM (%)	24.7	21.5	18.3	25.2	0.685
Glucose status (%)					<0.001
NGT	78.4	71.1	75.4	62.2	
IFG	6.2	0.8	11.5	2.6	
IGT	7.0	18.2	6.6	16.7	
Combined IFG/IGT	1.8	1.7	6.6	7.7	
T2DM	6.6	8.3	0.0	10.9	
Employment status (%)					0.429
Paid job	44.6	49.2	43.3	36.8	
Retired	39.7	37.5	36.7	42.6	
Other	15.6	13.3	20.0	17.0	
Education level (%)					0.257
Low	17.9	11.8	25.0	21.7	
Intermediate	31.8	39.5	28.3	30.9	
High	50.2	48.7	46.7	47.4	

Differences between tissue-specific IR groups were assessed using one-way ANOVA with Bonferroni post-hoc pairwise comparisons for numerical data (mean ± SD), and using Fisher's exact test for categorial data (%).
§ significantly different from No MIR/LIR (p<0.05)
† significantly different from MIR (p<0.05)
‡ significantly different from LIR (p<0.05)
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine transaminase; AST, aspartate aminotransferase; NGT, normal glucose tolerant; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.

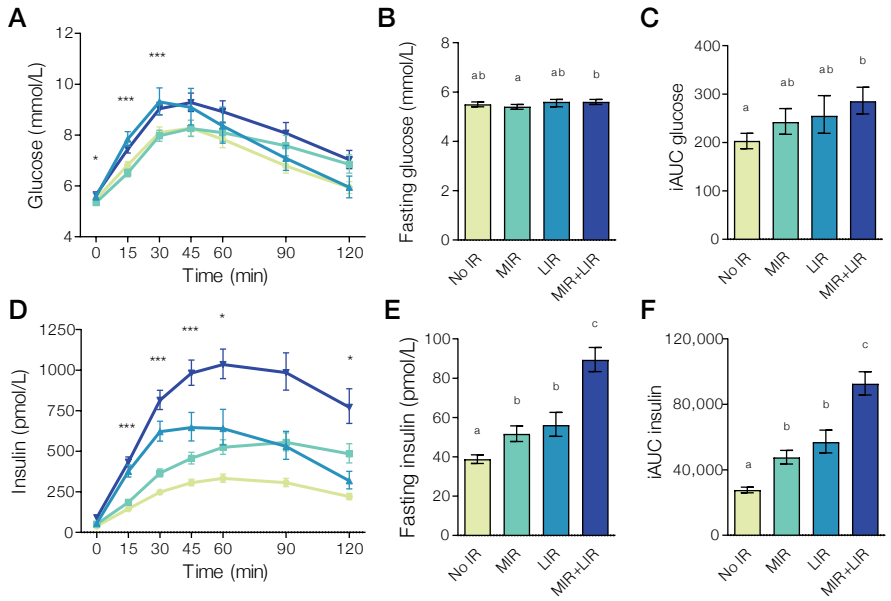

Figure 6. Plasma glucose (A–C) and insulin (D–F) concentrations during an oral glucose tolerance test according to insulin resistance phenotype. (A,D): data are geometric means with 95% confidence intervals; significant differences for MIR vs. LIR as analyzed using estimated marginal means from linear mixed-effects models with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons are denoted with *(p < 0.05) or ***(p < 0.001). (B,C,E,F): data are adjusted geometric means with 95% confidence intervals. Different letters (a, b, c, d) indicate significant differences (p < 0.05) between IR phenotypes, as tested using ANCOVA with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons.

Habitual Dietary Intake

FFQ data were available from 549 participants. After exclusion of data from 84 and 4 individuals due to energy under- and overreporting, respectively, data from 461 participants were included in the analyses. The proportion of misreporters did not differ between the IR phenotypes (p = 0.411). Energy intake tended to be lower in the MIR group compared to the other groups when adjusted for sex (Table 3; p = 0.062). Intake of energy from saturated fat was highest in the combined MIR/LIR group, although only statistically significantly higher compared to the MIR group. Other components of macronutrient composition of habitual dietary intake, expressed as en%, did not differ between the IR phenotypes when adjusted for sex. Alcohol consumption was lower in the combined MIR/LIR group compared to No MIR/LIR (overall p = 0.011).

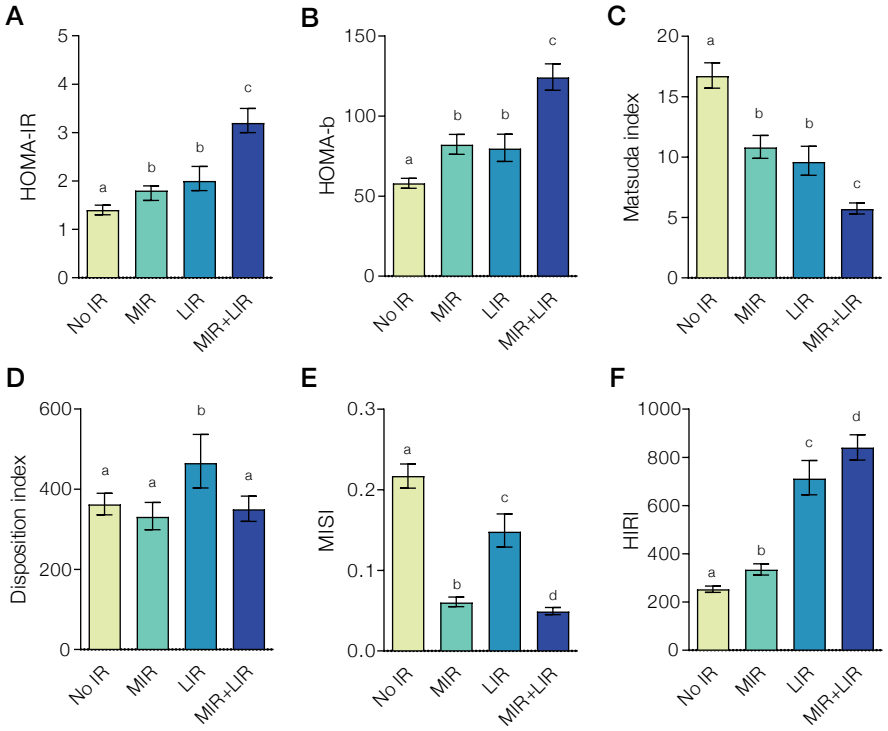

Figure 7. HOMA-IR (A), HOMA-β (B), Matsuda index (C), disposition index (D), muscle insulin sensitivity index (E), and hepatic insulin resistance index (F) according to insulin resistance (IR) phenotype. Data are adjusted geometric means with 95% confidence intervals. Different letters (a, b, c, d) indicate significant differences ($p < 0.05$) between IR phenotypes, as tested using ANCOVA with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons.

Table 3. Habitual dietary intake from FFQ according to insulin resistance phenotype

	No MIR/LIR (n=227)	MIR (n=121)	LIR (n=61)	Combined MIR/LIR (n=156)	*P*-value
Energy (MJ)[a]	9.5 ± 1.0	8.8 ± 1.0	9.5 ± 1.0	9.6 ± 1.0	0.062
Fat (en%)	37.6 ± 0.4	36.8 ± 0.6	37.3 ± 0.8	38.5 ± 0.5	0.127
Monounsaturated fat	13.5 ± 0.2	13.1 ± 0.2	13.3 ± 0.3	13.6 ± 0.2	0.551
Polyunsaturated fat	7.2 ± 0.1	7.1 ± 0.2	7.1 ± 0.2	7.1 ± 0.1	0.946
Saturated fat	13.8 ± 0.2	13.4 ± 0.3	13.8 ± 0.4	14.5 ± 0.3†	0.024
Carbohydrates (en%)	41.1 ± 0.5	42.6 ± 0.6	42.1 ± 0.8	40.9 ± 0.5	0.137
Mono- and disaccharides	19.0 ± 0.4	20.0 ± 0.6	19.8 ± 0.8	18.4 ± 0.5	0.150
Polysaccharides	22.1 ± 0.3	22.6 ± 0.4	22.3 ± 0.6	22.5 ± 0.4	0.748
Fiber (g/MJ)	2.6 ± 0.0	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	0.538
Alcohol (en%)[b]	2.3 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.6 ± 0.1§	0.011
Protein (en%)	15.7 ± 0.2	15.7 ± 0.2	15.2 ± 0.3	15.8 ± 0.2	0.401
Animal-based, % of total	58.4 ± 0.7	58.4 ± 1.0	57.1 ± 1.3	59.5 ± 0.8	0.475
Plant-based, % of total	41.6 ± 0.7	41.6 ± 1.0	42.9 ± 1.3	40.5 ± 0.8	0.481

Differences between tissue-specific IR groups were assessed using ANCOVA with adjustment for sex and with Bonferroni post-hoc pairwise comparisons (adjusted mean ± SE).
[a] data were logtransformed to improve normality and reported as geometric means
[b] a constant was added before logtransformation to eliminate zero values
§ significantly different from No MIR/LIR ($p<0.05$)
† significantly different from MIR ($p<0.05$)
FFQ, Food Frequency Questionnaire; MJ, megajoule; en%, energy percentage of total energy intake.

Discussion

The purpose of the present article was to describe the study design of the PERSON study and to present preliminary screening results. In the PERSON study, individuals are classified based on IR phenotype at baseline, and randomized to follow a hypothesized optimal or suboptimal diet according to their metabolic phenotype. This study is one of the first randomized double-blind controlled trials in the field of precision nutrition to investigate whether a dietary intervention based on tissue-specific insulin sensitivity improves metabolic health to a greater extent compared to a hypothesized suboptimal diet.

Dietary Intervention

Both intervention diets prescribed in this study are largely in line with the Dutch dietary guidelines of the Health Council of the Netherlands (36). Data from the FFQ indicated that the habitual dietary intake of our study population did not meet these guidelines. In particular, average fiber intake (2.6 g/MJ) was well below the recommended 3.4 g/MJ, and lower than the targeted fiber intake of 3 g/MJ and 4 g/MJ in the HMUFA and LFHP interventions diets, respectively. In addition, average intake of calories from saturated fat (14 en%) exceeded the <10 en% that is recommended. In our study, prescribed intake of saturated fat and mono- and disaccharides, which is similar between the two interventions diets, is lower than the average habitual intake. Therefore, we expect that on average, participants will benefit from both dietary interventions, regardless of their IR phenotype. Nevertheless, we hypothesize to find greater improvements in glucose homeostasis and related outcomes in study participants that follow the anticipated optimal compared to suboptimal diet.

The hypothesis that dietary macronutrient composition interacts with tissue-specific IR is supported by findings from recent studies. A *post-hoc* analysis of the CORDIOPREV-DIAB study indicated that individuals with predominant MIR had a greater improvement in disposition index on a 2-year Mediterranean diet, while individuals with predominant LIR benefitted more from a diet high in complex carbohydrates and low in fat (18). In addition, individuals with LIR have been shown to have a more detrimental fasting plasma lipid profile (13) and impaired postprandial lipoprotein metabolism following high-fat meals (70) compared to individuals with MIR, which suggests that a low-fat diet may be especially beneficial for individuals with LIR (71). Furthermore, findings from other studies indicate that a high protein diet and high fiber diet may have beneficial effects for individuals with LIR, as both high protein and high fiber diets have been shown to successfully reduce liver fat content (72–75). Liver fat accumulation has been related to decreased suppression of hepatic glucose production in some studies (74, 76), linking liver fat to LIR, although the cause-effect relationship remains to be established. Moreover, increased fiber intake has been shown to improve insulin sensitivity in individuals with IFG but not IGT (77). IFG is characterized mainly by impaired hepatic insulin sensitivity (78, 79), which is in line with observations in our study that individuals with IFG are most often characterized as LIR.

In addition, dietary fat quality may impact skeletal muscle lipid handling. In an acute study, meals high in saturated fat resulted in increased postprandial skeletal muscle fatty TAG extraction and/or reduced intramyocellular lipid turnover compared to meals high in unsaturated FAs in insulin resistant individuals, which was accompanied by a lower postprandial insulin sensitivity (80). Taken together, a "one-size-fits-all" approach with population-wide dietary guidelines may not be optimal for

metabolic health for all individuals. A diet targeting tissue-specific IR is expected to increase the effectiveness of dietary interventions with respect to improvements in glucose homeostasis.

Changes in macronutrient composition within the context of an isocaloric diet can improve risk factors for cardiometabolic diseases, independent of weight loss (81). The two diets implemented in the PERSON study differ in macronutrient composition, and are both matched to the participants' individual energy requirements in order to maintain weight stability during the dietary intervention. Throughout the study, participants' body weight is monitored weekly, and adjustments in absolute energy intake, but not diet composition, are made if needed to maintain body weight. We provide key food products, perform unannounced food records, and conduct weekly check-ins with skilled dieticians and researchers, together increasing the incentive to adhere to the diet and the possibility to assess dietary compliance.

Extensive and Detailed Phenotyping

A strength of the PERSON-study is the extensive and detailed phenotyping of the study participants before and after the dietary intervention. This allows us to comprehensively study the metabolic underpinnings of the metabolic response to the dietary intervention. Next to performing highly standardized metabolic phenotyping in a laboratory setting, we also collect data in free-living conditions. Furthermore, in a subgroup of the study population several additional measurements such as the gold-standard hyperinsulinemic-euglycemic clamp are performed, which allows us to investigate the mechanisms involved in the pathophysiology of tissue-specific IR as well as how these may be affected by the dietary intervention.

Next to detailed metabolic phenotyping, we also collect data on mood, perceived well-being, food preferences and cognitive function. There are indications that blood glucose levels may be an important determinant of mood and cognitive function (19, 21, 82, 83). Additionally, gut microbial profile, which can be modulated by dietary intake, is linked to cognitive function and mood via the gut-brain axis (84, 85). Hence, by improving glucose homeostasis and metabolic health with a dietary intervention, individuals may also experience short-term benefits related to mental and emotional well-being and performance. Such directly perceivable benefits are expected to motivate individuals to better adhere to dietary advice.

In addition, the large amount of collected data will allow for the application of computational techniques to elucidate the inter-individual differences in glucose homeostasis and derive new functional insights. Both mechanistic and data-driven computational modeling approaches have been employed to expand on the physiological properties underlying meal responses (6, 7, 86). The frequently-sampled time series of metabolites (e.g., glucose, insulin) from the OGTT and continuous glucose monitoring will be used to construct models of short-term postprandial

dynamics, facilitating the assessment of individuals' capacity to regulate glucose levels in response to a meal. The detailed phenotypic information can be integrated using machine-learning models to derive a comprehensive model of glucose homeostasis. The data generated in the PERSON study will enable such computational methods to progress the field of precision nutrition.

Preliminary Screening Data

Tissue-specific or whole-body IR (either MIR, LIR or combined) was prevalent in ~60% of the population, which is similar to the reported prevalence of 65% in DMS (16). The prevalence of LIR in this study was lower as compared to DMS (11 vs. 17%, respectively). This can possibly be partly explained by the higher proportion of women in the PERSON study compared to DMS (59 vs. 44%, respectively), since LIR is less prevalent in women than men. Sexual dimorphism in glucose homeostasis and IR is well-recognized and has been linked to differences in relation to hormonal status, lipid handling and inflammatory profile (87), but does require further investigation. These data emphasize that future analyses within the PERSON study should also take sex-specific effects into account.

As expected based on the formulas used to classify MIR and LIR, our preliminary screening data confirmed that both MIR and LIR are related to worse glucose homeostasis compared to individuals without MIR or LIR, in line with observations from DiOGenes and DMS (16, 22). Interestingly, however, the majority of individuals with MIR and LIR (71 – 75%) were classified as normal glucose tolerant. Classical cutoff values only including plasma glucose levels may fail to detect important metabolic impairments related to insulin action, especially in early stages of disease development, while these disturbances are well-known to be highly predictive for the development of cardiometabolic diseases later in life (88, 89). Identification of metabolic impairments at an early stage before the onset of dysglycemia creates an important window of opportunity to use lifestyle interventions such as dietary modulation in order to delay or prevent further glycemic deterioration and progression to cardiometabolic disease.

Conclusion

The PERSON study is one of the first double-blind, randomized trials in the field of precision nutrition to investigate the effects of a more personalized dietary intervention based on tissue-specific insulin resistance phenotype, on metabolic health outcomes at the functional and molecular level, mental performance and perceived well-being. The high prevalence of tissue-specific IR in adults with overweight and obesity highlights the relevance of investigating the effects of targeted dietary approaches in order to define more optimal diets to improve glucose homeostasis, thereby preventing or delaying the development of cardiometabolic diseases. The PERSON study is expected to contribute knowledge on the effectiveness of targeted nutritional strategies to the emerging field of precision nutrition and enhance the understanding of the complex etiology of generalized and tissue-specific IR.

Data Availability Statement

The datasets presented in this article are not readily available because the data are part of an ongoing study. Requests to access the datasets should be directed to Ellen Blaak, e.blaak@maastrichtuniversity.nl.

Ethics Statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of MUMC+, Maastricht, The Netherlands. The patients/participants provided their written informed consent to participate in this study.

Author Contributions

EF, GG, LA, and EB: obtained funding. AG, IT, KJ, GH, ES, SB, LW, DT, EF, GG, LA, and EB: concept development and study design. AG, IT, KJ, SB, DY, and LW: data collection. GH, ES, EF, GG, LA, and EB: study coordination. AG, IT, and BE: data analysis. AG and IT: writing manuscript. AG, IT, KJ, GH, ES, GG, LA, and EB: revising manuscript. All authors read and approved the final manuscript.

Funding

This project was organized by and executed under the auspices of TiFN, a public-private partnership on precompetitive research in food and nutrition (project code: 16NH04). Funding for this research was obtained from the Netherlands Organization for Scientific Research, DSM, FrieslandCampina, Danone Nutricia Research, and the Top Sector Agri & Food the Netherlands.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

We thank laboratory and supporting staff and students from UM and WUR for their invaluable assistance. We thank all study participants for their commitment.

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Supplementary Material

Table S1. Inclusion and exclusion criteria for the PERSON study

Inclusion criteria	• Men and women aged 40-75 y • BMI 25-40 kg/m^2 • Body weight stability for at least 3 months (no weight change >3kg) • Predominantly muscle (MIR) or liver (LIR) insulin resistant
Exclusion criteria	**Diseases** • Pre-diagnosis of type 1 or type 2 diabetes mellitus • Renal or hepatic malfunctioning (pre-diagnosis or determined based on alanine aminotransferase [ALAT], aspartate aminotransferase [ASAT] and creatinine values) • Major gastrointestinal diseases or major abdominal surgery • Cardiovascular diseases (e.g. heart failure) or cancer • High blood pressure (untreated >160/100 mmHg, drug-regulated >140/90 mmHg) • Diseases affecting glucose and/or lipid metabolism (e.g. pheochromocytoma, Cushing's syndrome, acromegaly) • Anemia defined as hemoglobin (Hb) men <8.5 and women <7.5 mmol/l • Diseases with a life expectation shorter than 5 years • Major mental disorders • Drug treated thyroid diseases (well-substituted hypothyroidism is allowed) **Medication** • Medication known to interfere with study outcomes (e.g. peroxisome proliferator-activated receptor-α [PPAR-α] or PPAR-γ agonists [fibrates], sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, repaglinide, nateglinide and insulin, chronic use of NSAIDs) • Use of anticoagulants other than acetylsalicyclic acid • Use of antidepressants (stable use ≥3 months prior to and during the study is allowed) • Use of statins (stable use ≥3 months prior to and during study allowed) • Use of β-blockers (only for the extensive phenotyping participants) • Chronic corticosteroids treatment (>7 consecutive days of treatment) • Use of antibiotics within 3 months prior to the study **Lifestyle** • Participation in regular sports activities (>4 hours per week) • Abuse of alcohol (alcohol consumption >14 units/week) and/or drugs (cannabis included) • Regular smoking (including use of e-cigarettes) **Other** • Pregnant or lactating women who are planning to become pregnant • Inability to comply with the study diet

Table S2. Ingredients and macronutrient composition of the high-fat mixed meal

	Full-fat milk	Whipped cream	Sugar	Whipped ice cream	Total per meal
Amount per meal (g)	125	70	5	150	350
Energy (kJ)	347.5	973.0	85	1387.5	2793.0
Protein (g)	4.5	1.5	0	5.6	11.6
Fat (g)	4.5	24.6	0	19.5	48.6
Saturated fat (g)	3.1	17.5	0	12.8	33.4
Carbohydrates (g)	5.9	2.2	5	34.5	47.5
Sugar (g)	5.9	2.2	5	31.5	44.5

Table S3. Overview of standardized products provided during at-home days and amounts provided per energy group

Meal moment (time frame)	Product	Nutrients per 100 g						Amounts (g) provided per energy group		
		Energy (kJ)	Fat (g)	Saturated fat (g)	Protein (g)	Carbohydrates (g)	Sugars (g)	6-8 MJ	9-11 MJ	12-13 MJ
Breakfast (7am-9am)	Drink yogurt	247	0.8	0.5	3.4	8.1	7.3	400	400	400
	Gingerbread	1304	1.1	0.4	2.9	69.6	37.1	28	28	28
Snack (10am-11am)	Raisin cake	1785	21.3	6.8	6.3	51.7	35.0	60	60	60
	Banana	401	0.3	0.1	1.1	20.6	15.5	130	130	130
	Apple juice	194	0.0	0.0	0.1	11.2	10.5	200	200	200
Lunch (12am-1pm)	Wheat bread	1000	1.8	0.4	9.8	42.9	2.0	56	84	112
	Cream cheese	1540	33.3	10.7	14.0	2.7	2.0	15	30	30
	Hazelnut spread	2347	35.3	9.3	6.0	54.0	50.0	15	15	30
	Semi-skimmed milk	192	1.5	1.0	3.4	4.7	4.7	200	200	200
	Yogurt with strawberry sauce	368	2.0	1.3	4.0	13.0	11.0	190	190	190
Snack (3pm-4pm)	Apple	254	0.2	0.0	0.3	13.0	10.4	135	135	135
	Potato chips	2261	33.2	5.7	6.4	52.5	1.4	28	28	28
	Lemonade	170	0.1	0.0	0.1	9.7	9.6	200	200	200
Dinner (6pm-7pm)	Macaroni meal	447	3.6	1.2	5.6	12.1	2.0	350	450	550

Nutrient composition was calculated using the 2016 Dutch Food Composition Table[90]
kJ, kilojoule; MJ, megajoule

Table S4. Macronutrient composition of standardized meal moments during home-days per energy group

Energy group	Meal moment (time frame)	Energy (kJ)	Fat (g)	Saturated fat (g)	Protein (g)	Carbohydrates (g)	Sugars (g)
6-8 MJ	Breakfast (7am-9am)	1353	3.5	2.1	14.4	51.9	39.6
	Snack (10am-11am)	1980	13.2	4.2	5.4	80.2	62.2
	Lunch (12am-1pm)	2226	18.1	7.7	22.8	66.6	39.2
	Snack (3pm-4pm)	1316	9.8	1.6	2.4	51.6	33.7
	Dinner (6pm-7pm)	1565	12.7	4.2	19.7	42.2	6.9
	Total	8440	57.3	19.8	64.7	292.5	181.6
9-11 MJ	Breakfast (7am-9am)	1353	3.5	2.1	14.4	51.9	39.6
	Snack (10am-11am)	1980	13.2	4.2	5.4	80.2	62.2
	Lunch (12am-1pm)	2738	23.6	9.4	27.6	79.1	40.1
	Snack (3pm-4pm)	1316	9.8	1.6	2.4	51.6	33.7
	Dinner (6pm-7pm)	2012	16.3	5.4	25.3	54.3	8.9
	Total	9399	66.4	22.7	75.1	317.1	184.5
12-13 MJ	Breakfast (7am-9am)	1353	3.5	2.1	14.4	51.9	39.6
	Snack (10am-11am)	1980	13.2	4.2	5.4	80.2	62.2
	Lunch (12am-1pm)	3369	29.4	10.9	31.2	99.2	48.2
	Snack (3pm-4pm)	1316	9.8	1.6	2.4	51.6	33.7
	Dinner (6pm-7pm)	2459	19.9	6.6	30.9	66.3	10.9
	Total	10477	75.8	25.4	84.3	349.2	194.6

Nutrient composition was calculated using the 2016 Dutch Food Composition Table[90]
kJ, kilojoule; MJ, megajoule

Table S5. Glucose homeostasis parameters derived from OGTT according to insulin resistance phenotype

	No MIR/LIR (n=227)	MIR (n=121)	LIR (n=61)	Combined MIR/LIR (n=156)	*P*-value
Fasting glucose (mmol/L)	5.5 (5.4, 5.6)	5.4 (5.3, 5.5)	5.6 (5.4, 5.7)	5.6 (5.5, 5.7)†	0.005
Fasting insulin (pmol/L)	38.7 (36.6, 41.0)	51.6 (47.8, 55.8)§	56.1 (50.4, 62.6)§	89.3 (83.4, 95.6)§†‡	<0.001
2-hr glucose (mmol/L)	5.9 (5.7, 6.2)	6.9 (6.5, 7.3)§‡	5.9 (5.5, 6.4)†	7.0 (6.6, 7.3)§‡	<0.001
2-hr insulin (pmol/L)	220.6 (200.3, 242.9)	488.8 (428.0, 557.9)§‡	316.3 (262.7, 381.0)§†	759.6 (676.2, 853.7)§†‡	<0.001
iAUC glucose (AU)	203 (187, 219)	242 (217, 270)	255 (219, 297)	285 (259, 314)§	<0.001
iAUC insulin (AU)	27612 (25918, 29409)	47512 (43522, 51822)§	56855 (50334, 64236)§	92554 (85757, 99915)§†‡	<0.001
HOMA-IR (AU)	1.4 (1.3, 1.5)	1.8 (1.6, 1.9)§	2.0 (1.8, 2.3)§	3.2 (3.0, 3.5)§†‡	<0.001
HOMA-β (AU)	58.0 (54.9, 61.2)	82.1 (76.2, 88.6)§	79.7 (71.7, 88.7)§	124.2 (116.2, 132.7)§†‡	<0.001
Matsuda index (AU)	16.7 (15.7, 17.8)	10.8 (9.9, 11.8)§	9.6 (8.5, 10.9)§	5.7 (5.3, 6.2)§†‡	<0.001
Disposition index (AU)	362 (336, 390)	331 (299, 367)‡	465 (403, 537)§†	350 (320, 383))‡	0.002
MISI (AU)	0.217 (0.202, 0.232)	0.060 (0.055, 0.067)§‡	0.148 (0.129, 0.170)§†	0.049 (0.045, 0.054)§†‡	<0.001
HIRI (AU)	253 (240, 266)	334 (312, 359)§‡	712 (645, 787)§†	840 (789, 894)§†‡	<0.001

Differences between tissue-specific IR groups were assessed using ANCOVA with adjustment for sex and Bonferroni post-hoc pairwise comparisons. Data were logtransformed to improve normality and reported as adjusted geometric means with 95% confidence interval.

§ significantly different from No MIR/LIR ($p<0.05$)

† significantly different from MIR ($p<0.05$)

‡ significantly different from LIR ($p<0.05$)

OGTT, oral glucose tolerance test; iAUC, incremental area under the curve; AU, arbitrary units; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; MISI, muscle insulin sensitivity; HIRI, hepatic insulin resistance.

5

Hepatic insulin resistance and muscle insulin resistance are characterised by distinct postprandial plasma lipid profiles

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In preparation

Abstract

Background: The pathophysiology of insulin resistance (IR) is characterised by great heterogeneity, with inter-individual differences in IR severity in the various metabolic organs. Tissue-specific IR predominantly in the muscle (muscle IR) or liver (liver IR) has previously been linked to differential fasting metabolite profiles, but postprandial metabolite profiles have not been investigated in tissue-specific IR yet.

Aim: Given the importance of postprandial metabolic impairments in the pathophysiology of cardiometabolic diseases, we compared fasting and postprandial metabolite profiles in response to a high-fat mixed meal between individuals with predominant muscle IR or liver IR.

Methods: This cross-sectional study included data from 214 women and men with BMI 25-40 kg/m^2, aged 40-75 years, and with predominant muscle IR or liver IR. Tissue-specific IR was assessed using the muscle insulin sensitivity index (MISI) and hepatic insulin resistance index (HIRI), which were calculated from the glucose and insulin responses during a 7-point oral glucose tolerance test (OGTT). Plasma samples were collected before (T=0) and after (T=30, 60, 120, 240 min) consumption of a high-fat mixed meal and 247 metabolites, including lipoproteins, apolipoproteins, cholesterol, triglycerides, ketone bodies, and amino acids, were quantified using nuclear magnetic resonance spectroscopy.

Results: Liver IR was characterised by greater increases in postprandial plasma levels of large and very large VLDL particles, and triacylglycerol (TAG) in large and very large VLDL, small and medium-sized LDL, and small HDL particles, compared to muscle IR. In liver IR, the postprandial plasma fatty acid (FA) profile consisted of a higher percentage of saturated FA, and a lower percentage of polyunsaturated FA, compared to muscle IR.

Conclusion: People with muscle IR or liver IR have distinct postprandial metabolite profiles, with more dyslipidemic postprandial metabolite responses in those with liver IR compared to muscle IR.

Introduction

Overweight, obesity and related metabolic complications and chronic diseases, such as cardiovascular disease (CVD) and type 2 diabetes (T2DM), are a massive burden to public health.[1] Insulin resistance (IR) is one of the earliest metabolic disturbances that underlies the development of many obesity-related metabolic complications.[2-4] Apart from its central role in glucose homeostasis, insulin is a major regulator of lipid and protein metabolism. As such, IR is commonly accompanied by lipid and lipoprotein abnormalities, although the causal and temporal relationships of these links are unclear.[5]

The pathophysiology of whole-body IR is characterised by great heterogeneity, with inter-individual differences in IR severity in the various metabolic organs, including the liver and skeletal muscle. Tissue-specific IR in liver and skeletal muscle has previously been linked to distinct plasma metabolite and lipidome profiles.[6,7] These findings may indicate that either the mechanisms causing tissue-specific IR differ between the affected tissues or that tissue-specific IR results in different metabolic disturbances. More specifically, muscle IR has been associated with lower fasting plasma concentrations of lysophosphatidylcholines, while liver IR has been associated with higher fasting plasma levels of triacylglycerols (TAG) and ketogenic amino acids, lower levels of ketone bodies, and higher diacylglycerols (DAG), the latter in women, but not in men.[6,7]

Furthermore, liver IR has been characterised by elevated postprandial total TAG in response to an oral fat load, compared to muscle IR and insulin-sensitive individuals.[8] Changes in postprandial metabolite levels reflect the complex interplay of the production, secretion, and clearance by the various metabolic organs, in particular the liver, adipose tissue, and skeletal muscle. Therefore, postprandial metabolite concentrations provide more insights into the metabolism and functioning of these key metabolic organs than fasting metabolite levels. Importantly, early metabolic perturbations are more likely to become apparent in the postprandial state, when complex processes in these tissues act to maintain or regain homeostasis. Accordingly, postprandial metabolites, including TAGs, in the circulation are important predictors of risk for future CVD and related metabolic diseases, independent of fasting measures.[9-11]

To gain a better understanding of fasting and postprandial metabolism in tissue-specific IR, we compared fasting and postprandial metabolite profiles, including lipoproteins, apolipoproteins, cholesterol, triglycerides, ketone bodies, and amino acids in response to a high-fat mixed meal in individuals with predominant muscle IR or liver IR.

Methods

Study design and participants

This study is a cross-sectional analysis using baseline data from the PERSonalised Glucose Optimization Through Nutritional Intervention (PERSON) study, a two-centre, randomised, dietary intervention trial that was conducted from May 2018 until November 2021 at Maastricht University Medical Center+ (MUMC+) and Wageningen University (WUR) in the Netherlands. The design and methodology have been described in detail previously.[12] The trial was performed in line with the principles of the Declaration of Helsinki, approved by the Medical Ethical Committee of the MUMC+ (NL63768.068.17), and registered at ClinicalTrials.gov (NCT03708419). All participants gave written informed consent.

Participants were recruited via a volunteer database, flyers, and local newspaper and online media advertisements. Inclusion criteria were: age 40–75 years, BMI 25–40 kg/m^2, body weight stability for at least three months (no weight gain or loss >3 kg), and tissue-specific IR, characterised as predominant muscle or liver IR. Exclusion criteria included pre-diagnosis of type 2 diabetes, diseases or medication use that affect glucose or lipid metabolism, major gastrointestinal disorders, history of major abdominal surgery, uncontrolled hypertension, smoking, alcohol consumption >14 units/wk, and >4 h/wk moderate-to-vigorous physical activity. Using statins was not an exclusion criterium in the original trial because we did not expect interference with the primary study outcomes. However, statin users were excluded from the current analysis due to statins' effects on fasting and postprandial cholesterol and triglycerides.[13,14] Data on demographics, medical history, family history of DM (≥1 first-degree relative with DM), and medication use and lifestyle were collected by questionnaire.

Tissue-specific insulin resistance

Details on the assessment of eligibility have been described previously.[15] Tissue-specific IR was assessed at screening and baseline using the plasma glucose and insulin concentrations during a 7-point OGTT. After an overnight fast, participants ingested 200 ml of a 75 g glucose drink (Novolab) within 5 minutes. Blood samples were collected from the antecubital vein via intravenous cannula before (t = 0 min) and after ingestion of the glucose drink (t = 15, 30, 45, 60, 90, and 120 min). Plasma glucose and insulin concentrations were quantified by enzymatic assay or enzyme-linked immunoassay (ELISA), respectively, and used for calculation of the muscle insulin sensitivity index (MISI) and hepatic insulin resistance index (HIRI).[16,17] Tertile cut-offs for MISI and HIRI from a previous study with a similar study population[6,18] were used to identify individuals with predominant muscle IR or liver IR. Baseline measurements were performed within three months after screening. In this study, MISI and HIRI from the screening and baseline measurements were averaged.

The Matsuda index, a measure of whole-body insulin sensitivity, was calculated using glucose and insulin values from time points 0, 30, 60, 90, and 120 minutes: *(10,000 ÷ square root of [fasting plasma glucose in mmol/L × fasting insulin in mU/L] × [mean glucose in mmol/L x mean insulin in mU/L])*.[19] Glucose status was defined according to WHO criteria[20]: normal glucose tolerance (NGT), fasting glucose <6.1 mmol/L and 2-hour glucose <7.8 mmol/L; impaired fasting glucose (IFG), fasting glucose 6.1 – 6.9 mmol/L and 2-hour glucose <7.8 mmol/L; impaired glucose tolerance (IGT), fasting glucose <6.1 mmol/L and 2-hour glucose 7.8 – 11.0 mmol/L; combined IFG/IGT, fasting glucose 6.1 – 6.9 mmol/L and 2-hour glucose 7.8-11.0 mmol/L; T2DM, fasting glucose ≥7.0 mmol/L and/or 2-hour glucose ≥11.1 mmol/L.

High-fat mixed-meal test

After a 12-hour overnight fast, participants visited the facilities for a high-fat mixed-meal test. The evening before the visit, participants consumed a standardised low-fat pasta meal (30% of energy intake [en%] fat, 49 en% CHO, 21 en% protein; 1,560–2,460 kJ, depending on estimated energy requirements), and they were instructed to refrain from alcohol and vigorous physical activities for three days before the visit. The liquid high-fat mixed meal was prepared in the metabolic kitchen using ice cream, full-fat milk, whipped cream, and sugar. It contained 49 g fat (33 g SFA), 48 g carbohydrates, and 12 g protein (Table 1).

Table 1. Nutrient composition of the high-fat mixed meal

	Ice cream	Full-fat milk	Whipped cream	Sugar	Total per meal
Amount per meal, g	150	125	70	5	350
Energy, kJ	1388	348	973	85	2793
Protein, g	5.6	4.5	1.5	0	11.6
Fat, g	19.5	4.5	24.6	0	48.6
Saturated fat, g	12.8	3.1	17.5	0	33.4
Carbohydrates, g	34.5	5.9	2.2	5.0	47.5
Sugar, g	31.5	5.9	2.2	5.0	44.5

An intravenous cannula was inserted in the antecubital vein, and a fasting blood sample was drawn at least 30 minutes after insertion. Participants consumed the meal within 5 minutes. Postprandial blood samples were drawn at t = 30, 60, 90, 120, 180, and 240 minutes.

Glucose and insulin levels were measured in EDTA plasma from timepoints 0, 30, 60, 120, 180, and 240 min by enzymatic assay or ELISA, respectively. Fasting plasma NEFA and fasting serum TAG, total cholesterol, and HDL cholesterol were quantified

with enzymatic assays. The inflammatory marker C-reactive protein (CRP) was measured in fasting plasma with a Luminex immunoassay.

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as *(fasting glucose in mmol/L × fasting insulin in mU/L) ÷ 22.5,* and HOMA of β-cell function (HOMA- β) was calculated as *(20 × fasting insulin in mU/L) ÷ (fasting glucose in mmol/L – 3.5).*[21] Adipose tissue IR (Adipo-IR) was estimated by calculating (*fasting insulin in pmol/L x fasting NEFA in mmol/L).*

Fasting and postprandial metabolite profile

Metabolite concentrations were quantified in plasma samples from T = 0, 30, 60, 120, and 240 min by the Nightingale high-throughput nuclear magnetic resonance (NMR) metabolomics platform (Nightingale Health Ltd., Helsinki, Finland)[22,23]. This platform provides quantitative data on 164 metabolites, including 14 lipoprotein subclasses, their lipid concentrations and composition, apolipoprotein A-I and B, major fatty acids, (branched-chain) amino acids, glycolysis-related measures, and ketone bodies. In addition, it provides data on three lipoprotein sizes (VLDL, LDL, and HDL diameter) and 82 relative measures (i.e. percentages, ratios). We used clinically measured plasma glucose rather than NMR-measured glucose and excluded the measure 'Unsaturation', assessing a total of 247 metabolic measures.

The postprandial net incremental area under the curves (iAUC) were calculated using the trapezoid method.[24] For the calculation of iAUCs, metabolite curves from participants were excluded if values of ≥2 time points were missing (n = 2) and/or if the last (t = 240 min) value was missing (n = 5). For metabolite curves with one missing value at 30-120 minutes, the missing values were imputed with the weighted metabolite average of the two closest time points of that particular metabolite of that participant (n = 13).

Anthropometrics, body composition and ectopic fat

Waist and hip circumference were measured in duplicate using a non-flexible measuring tape. Whole-body and regional fat mass (i.e. android and gynoid fat mass) were assessed using dual-energy X-ray absorptiometry (DXA) (WUR, Lunar Prodigy, GE Healthcare; MUMC+, Discovery A, Hologic). Intrahepatic lipid content was quantified after a ≥2-hour fast with a 3T magnetic resonance imaging (MRI) scanner using proton magnetic resonance spectroscopy (^{1}H-MRS) (WUR) or a 6-minute whole-body MRI scan protocol and automated image analysis (MUMC+) (AMRA Medical AB, Linköping, Sweden) . Visceral adipose tissue (VAT) volume was also quantified in MUMC+ participants from the MRI. Details of these methods have been described previously.[12]

Habitual dietary intake and physical activity

Habitual dietary intake was assessed with a validated 163-item semi-quantitative food frequency questionnaire (FFQ)[25]. Diet quality was assessed with the Dutch Healthy Diet index 2015 (DHD15-index)[26], which is a score between 0 (no adherence) and 150 (complete adherence) that reflects adherence to the Dutch dietary guidelines. Self-reported habitual physical activity was assessed with the Baecke questionnaire[27].

Statistical analyses

Baseline characteristics were compared between IR phenotypes and between men and women using an independent t-test for normally distributed numerical data, a Mann-Whitney test for non-normally distributed numerical data, and using Fisher's exact test for categorical data.

Differences in fasting plasma metabolite levels and postprandial metabolite iAUCs between muscle IR and liver IR were tested using ANCOVA with adjustment for age, sex, study centre, BMI, and waist-to-hip ratio. In addition, because the iAUC does not capture postprandial metabolite dynamics and iAUCs may be similar for postprandial curves with a different shape, we also examined differences in postprandial responses between the IR phenotypes using linear mixed models with the absolute metabolite response (postprandial metabolite concentration minus the fasting value) as the dependent variable, the postprandial time points as repeated measures, and adjustment for age, sex, study centre, BMI, and waist-to-hip ratio. LSD post-hoc pairwise comparisons were performed if the overall p-value was significant. Associations between MISI/HIRI and fasting plasma metabolites and postprandial metabolite iAUCs were tested using linear regression analyses with adjustment for age, sex, study centre, BMI, waist-to-hip ratio and HIRI/MISI. Since sex-specific associations between tissue-specific IR and fasting plasma metabolites have been reported previously,[6,7] we tested for effect modification by sex by testing interactions between IR phenotype or MISI/HIRI and sex. For the linear regression analyses and ANCOVA, fasting metabolites, metabolite iAUCs, MISI and HIRI were log-transformed (log2) and autoscaled to allow for direct comparison of effect sizes.

Because many of the 247 metabolite measures are highly correlated, we estimated the number of independent tests performed by calculating how many principal components explained 95% of the variation in the data. Nineteen principal components explained 95% of the variation in the data. Therefore, raw p-values were multiplied by 38 to account for performing two sets of analyses (fasting and postprandial metabolites) in 19 independent metabolite measures.

Results

Data on plasma metabolomics were available from 230 participants; 142 individuals with muscle IR and 88 with liver IR. Sixteen participants were excluded from analyses due to statin use (muscle IR, 7.7%, n = 11; liver IR, 5.7%, n = 5), resulting in 131 individuals with muscle IR and 83 individuals with liver IR that were included in the analyses.

Baseline characteristics

Table 1 shows anthropometrics, body composition, glucose homeostasis, cardio-metabolic parameters, medical history, and lifestyle factors according to IR phenotype and sex. The participants' mean (± SD) age was 60 ± 8 years, and 61% were women. Individuals with liver IR had higher BMI and waist circumference and lower plasma CRP than those with muscle IR. In women only, VAT mass was higher in liver compared to muscle IR. In line with the calculations of MISI and HIRI, fasting plasma glucose and insulin levels were higher in liver IR, which was due to differences in women only, and plasma glucose and insulin two hours after the oral glucose load were higher in muscle IR, which was due to differences in men only.

Glucose and insulin responses to the mixed meal in muscle IR versus liver IR

After consumption of the mixed meal, plasma glucose levels in muscle IR were higher one and two hours post-meal compared to liver IR ($p_{curve} < 0.001$) (Fig. 1A). Plasma insulin levels were higher in liver compared to muscle IR in the first hour, and were lower two hours post-meal ($p_{curve} < 0.001$). Total iAUCs of glucose ($p_{iAUC} = 0.26$) and insulin ($p_{iAUC} = 0.75$) did not differ between the IR phenotypes (Fig. 1B).

Fasting plasma metabolites in liver IR and muscle IR

We compared fasting plasma metabolites between individuals with muscle IR and liver IR and examined associations of MISI and HIRI with fasting plasma metabolite concentrations (Table S1). All analyses with a significant interaction between IR phenotype or MISI/HIRI and sex were performed with stratification for sex.

None of the 164 absolute plasma metabolite concentrations and one of the 82 relative fasting metabolite measures differed between muscle and liver IR in the fasting state (Fig. 2, Fig. S1). For the significant relative metabolite, a significant sex interaction was observed. The VLDL, LDL, and HDL particle sizes did not differ between IR phenotypes in the fasting state. The relative fasting metabolite measure that differed between the IR phenotypes was the percentage of saturated fatty acid of total plasma FA (SFA%), which was higher in muscle IR (geometric mean 33.5%, 95% CI 33.2 to 33.9) compared to liver IR (32.5%, 32.1 to 32.9; $p = 0.011$), in men only (Fig. S1 and Table S1). Additional adjustment for habitual dietary intake of fat, SFA, LA, or carbohydrates did not affect this finding (data not shown).

Associations between MISI and HIRI with fasting plasma metabolites

Both MISI and HIRI were not significantly associated to any of the 164 absolute plasma metabolite concentrations after adjustment for multiple testing. Out of the 82 relative metabolite measures, MISI was associated with one metabolite measure and HIRI with none. Both indices were not associated with the three particle sizes. The one metabolite that MISI was positively associated with was the percentage of linoleic acid (LA%) of total plasma FA, but only in women (std. β 0.320, 0.167 to 0.490, $p = 0.004$) (Fig. S1, Table S1). Additional adjustment for habitual dietary intake of fat, SFA, LA, or carbohydrates did not affect this association (data not shown).

Postprandial metabolite responses in liver IR and muscle IR

Next, we compared the postprandial plasma metabolite responses between individuals with muscle IR and liver IR by testing differences in the iAUCs and postprandial curves after the mixed meal between the IR phenotypes. In addition, we examined associations of MISI and HIRI with postprandial plasma metabolite iAUCs. Analyses with a significant interaction between IR phenotype or MISI/HIRI and sex were performed with stratification for sex. All results can be found in Table S2.

Twenty-five of the 164 absolute metabolite iAUCs differed significantly between muscle and liver IR, of which six only in women (Fig. 2). Nine of the 82 relative metabolite iAUCs differed significantly between muscle and liver IR, of which one only in women (Fig. 2). VLDL, LDL, and HDL particle sizes did not differ postprandially timepoints between IR phenotypes.

As for the postprandial metabolite curves, three absolute and six relative postprandial metabolite curves differed between the IR phenotypes, the former in women only (Fig. S4, Table S3).

Of the 25 absolute metabolite iAUCs that differed between the IR phenotypes, most were higher in liver compared to muscle IR and included the TAG fraction of several lipoprotein subclasses. Postprandial concentrations of XL VLDL ($p_{iAUC} = 0.004$) and L VLDL ($p_{iAUC} < 0.001$) particles and their TAG and cholesterol content (all $p < 0.025$) were higher in liver compared to muscle IR (Figures 3 and S2-3). Furthermore, total TAG ($p_{iAUC} = 0.001$), and TAG in VLDL ($p_{iAUC} = 0.001$), LDL ($p_{iAUC} = 0.035$), M LDL ($p_{iAUC} = 0.006$), S LDL ($p_{iAUC} = 0.003$), and S HDL ($p_{iAUC} = 0.051$) were higher in liver IR compared to muscle IR (Figures 3 and S3). For the postprandial curves, the increase in TAG% in L VLDL was larger in muscle compared to liver IR ($p_{curve} = 0.047$) four hours post-meal (Fig. S4, Table S3).

Table 1. Demographic, clinical and metabolic characteristics of the study population according to IR phenotype and sex

	Muscle IR (n=131)	Liver IR (n=83)	*P*	Muscle IR – women (n=84)	Liver IR – women (n=46)	*P*	Muscle IR – men (n=47)	Liver IR – men (n=37)	*P*
Age, years	60 ± 8	59 ± 7	0.33	59 ± 8*	59 ± 8	0.98	63 ± 8*	60 ± 6	**0.028**
Women, n (%)	84 (64.1%)	46 (55.4%)	0.25						
Post-menopausal, n (%)				71 (84.5%)	38 (82.6%)	0.81			
BMI, kg/m^2	29.6 ± 3.3	30.7 ± 3.8	**0.033**	29.9 ± 3.5	31.0 ± 4.2	0.10	29.0 ± 2.9	30.2 ± 3.2	**0.010**
Waist circumference, cm	101.0 ± 8.8	103.8 ± 10.8	**0.034**	98.0 ± 8.3*	100.6 ± 10.4*	0.12	106.3 ± 6.9*	107.9 ± 9.9*	0.40
Body composition									
Body fat, %	38.3 ± 7.5	37.0 ± 7.0	0.20	42.7 ± 4.6*	42.0 ± 4.1*	0.36	30.5 ± 4.5*	30.8 ± 4.5*	0.69
Android fat, %	10.2 ± 1.8	10.2 ± 1.7	0.90	9.3 ± 1.4*	9.3 ± 1.4*	0.87	11.8 ± 1.4*	11.3 ± 1.3*	0.11
Gynoid fat, %	15.9 ± 2.1	15.7 ± 2.0	0.44	17.0 ± 1.6*	16.6 ± 1.6*	0.19	13.9 ± 1.5*	14.5 ± 1.9*	0.13
VAT, L[a]	5.4 ± 2.2	6.1 ± 2.1	0.12	4.0 ± 1.2*	4.9 ± 1.6*	**0.026**	7.3 ± 1.7*	7.4 ± 1.9*	0.84
VAT, cm^2 [b]	163 [119, 226]	176 [135, 200]	0.34	129 [110, 170]*	142 [128, 178]*	0.71	229 [191, 259]*	202 [174, 284]*	0.49
Liver fat, %[a]	4.9 [2.6, 9.9]	5.7 [3.3, 14.8]	0.92	5.4 [2.6, 11.7]	5.1 [3.4, 14.5]	0.87	4.4 [2.6, 10.1]	6.6 [3.3, 15.0]	1.00
Liver fat, %[b]	4.2 [1.4, 8.2]	2.4 [1.0, 5.2]	0.27	2.6 [1.3, 7.5]*	1.9 [0.9, 4.9]	0.42	5.5 [2.0, 9.1]*	3.6 [1.7, 6.6]	0.25
Glucose homeostasis									
Fasting glucose, mmol/L	5.3 [5.0, 5.6]	5.5 [5.1, 5.8]	**0.014**	5.1 [4.9, 5.8]*	5.5 [5.0, 5.8]	**0.008**	5.4 [5.2, 5.8]*	5.4 [5.1, 5.8]	0.84
2-hour glucose, mmol/L	6.5 [5.4, 7.7]	5.7 [4.8, 6.9]	**0.002**	6.5 [5.6, 7.4]	5.9 [4.8, 7.0]	0.10	6.5 [5.3, 8.5]	5.4 [4.7, 6.1]	**0.006**
Fasting insulin, pmol/L	44.7 [37.2, 60.1]	50.5 [41.0, 69.3]	**0.012**	43.0 [37.3, 56.4]	51.4 [38.9, 70.4]	**0.042**	46.0 [35.6, 63.3]	48.5 [43.2, 69.3]	0.25
2-hour insulin, pmol/L	403.3 [264.5, 585.6]	309.5 [192.8, 571.6]	**0.017**	407.0 [263.1, 593.8]	337.2 [231.3, 645.5]	0.40	403.3 [262.8, 583.2]	275.3 [176.3, 493.2]	**0.009**
HOMA-IR, AU	1.7 [1.3, 2.1]	1.9 [1.3, 2.5]	0.08	1.7 [1.2, 2.1]	2.1 [1.3, 2.8]	**0.02**	1.8 [1.3, 2.4]	1.7 [1.3, 2.3]	0.91
HOMA-β, AU	76.4 [62.5, 96.1]	82.4 [64.5, 98.8]	0.54	79.3 [63.3, 100.5]	76.7 [63.5, 109.7	0.98	74.5 [62.2, 90.0]	87.9 [67.0, 96.5]	0.23
Matsuda index, AU	4.8 [3.5, 6.7]	4.2 [3.0, 6.5]	0.18	5.0 [3.6, 7.1]	3.8 [2.8, 5.9]	**0.030**	4.5 [3.4, 6.2]	4.8 [3.3, 6.7]	0.49
Adipo-IR, AU	21.7 [15.0, 30.0]	23.7 [16.1, 39.4]	0.14	23.1 [16.4, 35.3]*	25.0 [17.3, 41.3]	0.30	18.9 [12.5, 26.1]*	21.2 [14.7, 35.2]	0.16
MISI, AU	0.096 [0.068, 0.130]	0.135 [0.104, 0.183]	**<0.001**	0.100 [0.066, 0.134]	0.125 [0.103, 0.200]	**<0.001**	0.093 [0.071, 0.117]	0.138 [0.108, 0.175]	**<0.001**
HIRI, AU	356 [284, 432]	601 [467, 716]	**<0.001**	359 [285, 441]	622 [499, 715]	**<0.001**	347 [284, 399]	565 [423, 750]	**<0.001**
Glucose status, n (%)			0.25			0.46			0.40
NGT	101 (77.1%)	65 (78.3%)		68 (81.0)	36 (78.3%)		33 (70.2%)	29 (78.4%)	
IGT	18 (13.7%)	6 (7.2%)		10 (11.9%)	4 (8.7%)		8 (17.0%)	2 (5.4%)	
IFG	2 (1.5%)	5 (6.0%)		1 (1.2%)	3 (6.5%)		1 (2.1%)	2 (5.4%)	
IGT+IFG	8 (6.1%)	5 (6.0%)		4 (4.8%)	3 (6.5%)		4 (8.5%)	2 (5.4%)	
T2DM	2 (1.5%)	2 (2.4%)		1 (1.2%)	0		1 (2.1%)	2 (5.4%)	

Table 1. Continued

	Muscle IR (n=131)	Liver IR (n=83)	*P*	Muscle IR – women (n=84)	Liver IR – women (n=46)	*P*	Muscle IR – men (n=47)	Liver IR – men (n=37)	*P*
Cardiometabolic parameters									
Fasting TAG, mmol/L	1.3 [1.0, 1.7]	1.5 [1.0, 1.9]	0.22	1.3 [1.0, 1.7]	1.4 [1.2, 1.9]	0.31	1.3 [1.0, 1.9]	1.5 [1.0, 1.9]	0.51
Fasting hypertriglyceridemia, n (%)	37 (28.2%)	30 (36.1%)	0.29	22 (26.2%)	17 (37.0%)	0.24	15 (31.9%)	13 (35.1%)	0.82
HDL cholesterol, mmol/L	1.3 ± 0.3	1.3 ± 0.3	0.34	1.4 ± 0.3*	1.4 ± 0.3*	0.97	1.1 ± 0.2*	1.1 ± 0.2*	0.53
Total cholesterol, mmol/L	5.4 ± 1.0	5.5 ± 1.0	0.39	5.6 ± 1.0*	5.7 ± 1.0*	0.41	5.1 ± 0.9*	5.3 ± 0.9*	0.39
Fasting NEFA, mmol/L	0.50 ± 0.17	0.48 ± 0.16	0.42	0.56 ± 0.18*	0.52 ± 0.16*	0.22	0.40 ± 0.11*	0.44 ± 0.15*	0.24
CRP, mg/L	1.4 [0.6, 2.5]	1.0 [0.5, 2.1]	**0.045**	1.7 [0.9, 2.8]*	1.4 [0.6, 2.7]*	0.35	1.1 [0.5, 2.0]*	0.7 [0.3, 1.2]*	0.11
Medical history									
Medication use, n (%)									
Antidepressants	7 (5.3%)	6 (7.2%)	0.57	7 (8.3%)*	3 (6.5%)	1.00	0*	3 (8.1%)	0.08
Antihypertensives	24 (18.3%)	10 (12.0%)	0.25	11 (13.1%)	5 (10.9%)	0.79	13 (27.7%)	5 (13.5%)	0.18
Anti-inflammatory	15 (11.5%)	4 (4.8%)	0.14	10 (11.9%)	2 (4.3%)	0.21	5 (10.6%)	2 (5.4%)	0.46
Other	41 (31.3%)	24 (28.9%)	0.76	29 (34.5%)	13 (28.3)	0.56	12 (25.5%)	11 (29.7%)	0.81
Family history of DM, n (%)	29 (22.1%)	18 (21.7%)	1.00	20 (23.8%)	11 (23.9%)	1.00	9 (19.1%)	7 (18.9%)	1.00
Lifestyle factors									
DHD2015-index, score	85.7 ± 15.4	81.3 ± 15.1	**0.044**	85.9 ± 16.8	85.2 ± 14.6*	0.82	85.4 ± 12.5	76.6 ± 14.6*	**0.005**
Habitual fat intake, en%	37.3 ± 5.5	37.2 ± 6.5	0.87	37.5 ± 5.2	39.2 ± 6.7*	0.12	37.1 ± 6.0	34.8 ± 5.5*	0.08
Habitual SFA intake, en%	13.6 ± 2.5	13.9 ± 3.1	0.48	13.7 ± 2.6	14.6 ± 3.5*	0.16	13.4 ± 2.3	13.0 ± 2.4*	0.54
Habitual sugar intake, en%	19.8 ± 5.1	19.2 ± 6.7	0.44	20.3 ± 5.4	19.9 ± 6.5	0.67	18.9 ± 4.4	18.3 ± 6.8	0.61
Habitual alcohol consumption, g	5.0 [1.7, 10.3]	6.1 [1.1, 14.7]	0.47	4.0 [1.0, 9.9]*	2.6 [0.1, 10.1]*	0.33	6.9 [3.1, 11.5]*	13.1 [3.7, 21.5]*	0.08
Habitual physical activity, Baecke score	8.2 ± 1.1	8.4 ± 1.3	0.35	8.3 ± 1.2	8.7 ± 1.2*	0.06	8.2 ± 1.0	8.1 ± 1.4*	0.60

Differences between IR phenotypes were assessed using independent t-test for normally distributed numerical data (mean ± SD), Mann-Whitney test for non-normally distributed numerical data (median [25th percentile, 75th percentile], and using Fisher's exact test for categorical data (n [%]).*p<0.05 for the difference between women and men within IR phenotype group. BMI, body mass index; VAT, visceral adipose tissue; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA- β, homeostatic model assessment of β-cell function; Adipo-IR, adipose tissue insulin resistance; MISI, muscle insulin sensitivity index; HIRI, hepatic insulin resistance index; NGT, normal glucose tolerant; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; T2DM, type 2 diabetes mellitus; TAG, triacylglycerol; NEFA, non-esterified fatty acids; CRP, C-reactive protein; DHD2015-index, Dutch Healthy Diet 2015 index; SFA, saturated fatty acids.

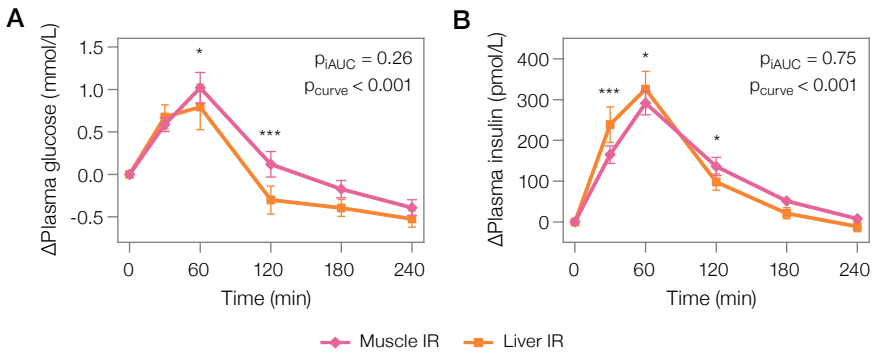

Figure 1. Plasma glucose (A) and insulin (B) responses to consumption of the high-fat mixed meal in individuals with liver IR and muscle IR. Responses were defined as change from fasting (value at postprandial timepoint – fasting value), and data are shown as means with 95% confidence intervals. Differences between liver IR and muscle IR were tested using linear mixed-effects models with adjustment for age, sex, centre, BMI, and waist-to-hip ratio. Significant LSD post-hoc pairwise comparisons per timepoint are denoted with *($p < 0.05$) or ***($p < 0.001$).

Postprandial plasma fatty acid profiles also differed between the IR phenotypes, with higher postprandial iAUCs of both total MUFA (p_{iAUC} = 0.012) and total SFA (p_{iAUC} = 0.028) in liver compared to muscle IR (Figures 5 and S3). Postprandial total PUFA% was lower in liver compared to muscle IR (p_{iAUC} = 0.025), as were percentages of the PUFAs omega-6 FA (p_{iAUC} = 0.021), and LA (p_{iAUC} = 0.027) (Figures 5 and S3). Postprandial SFA% tended to be higher (p_{iAUC} = 0.065) in liver compared to muscle IR. Additional adjustment for habitual dietary intake of fat, SFA, or LA resulted in a significant difference in postprandial SFA% between the IR phenotypes. (p_{iAUC} < 0.04). This additional adjustment did not affect other results (data not shown).

Seven of the 45 observed differences in postprandial metabolites between the IR phenotypes were found in women only. These include higher postprandial TAG in M VLDL (p_{iAUC} = 0.015), cholesterol (p_{iAUC} = 0.039) and cholesteryl esters in M LDL (p_{iAUC} = 0.016), and cholesteryl esters in S LDL (p_{iAUC} = 0.035) in liver compared to muscle IR (Fig. S2). In addition, postprandial MUFA% was higher in liver compared to muscle IR (p_{iAUC} = 0.027). As for the postprandial metabolite curves, the postprandial increases in cholesterol (p_{curve} = 0.029), free cholesterol (p_{curve} = 0.050), and cholesteryl esters (p_{curve} = 0.021) in chylomicrons and XXL VLDL, were higher four hours post-meal in liver IR compared to muscle IR (Fig. S4, Table S3).

Postprandial (branched-chain) amino acids, ketone bodies, glycolysis-related metabolites, or other metabolites did not differ between muscle IR and liver IR (Fig. 5).

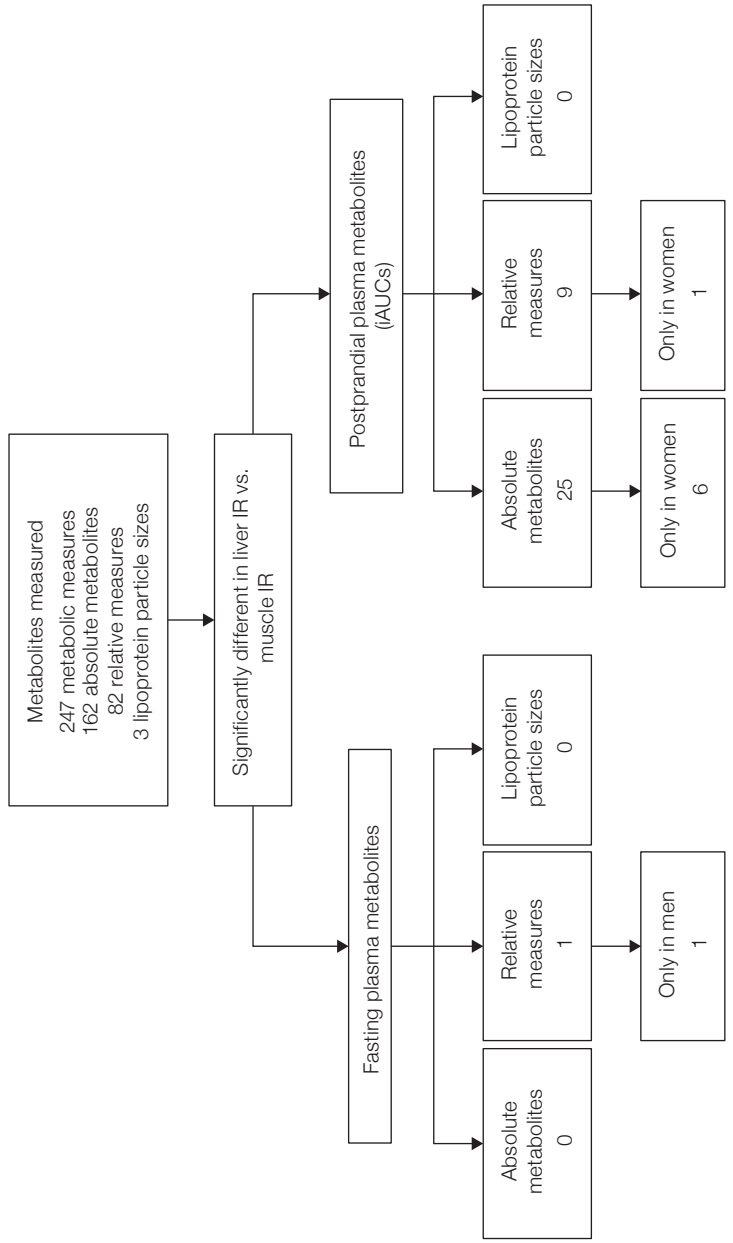

Figure 2. Flow diagram of the number of fasting and postprandial metabolites that were significantly different between individuals with liver or muscle IR. Differences between liver IR and muscle IR were tested using ANCOVA with adjustment for age, sex, centre, BMI, and waist-to-hip ratio. P-values were adjusted for multiple testing using a Bonferroni correction.

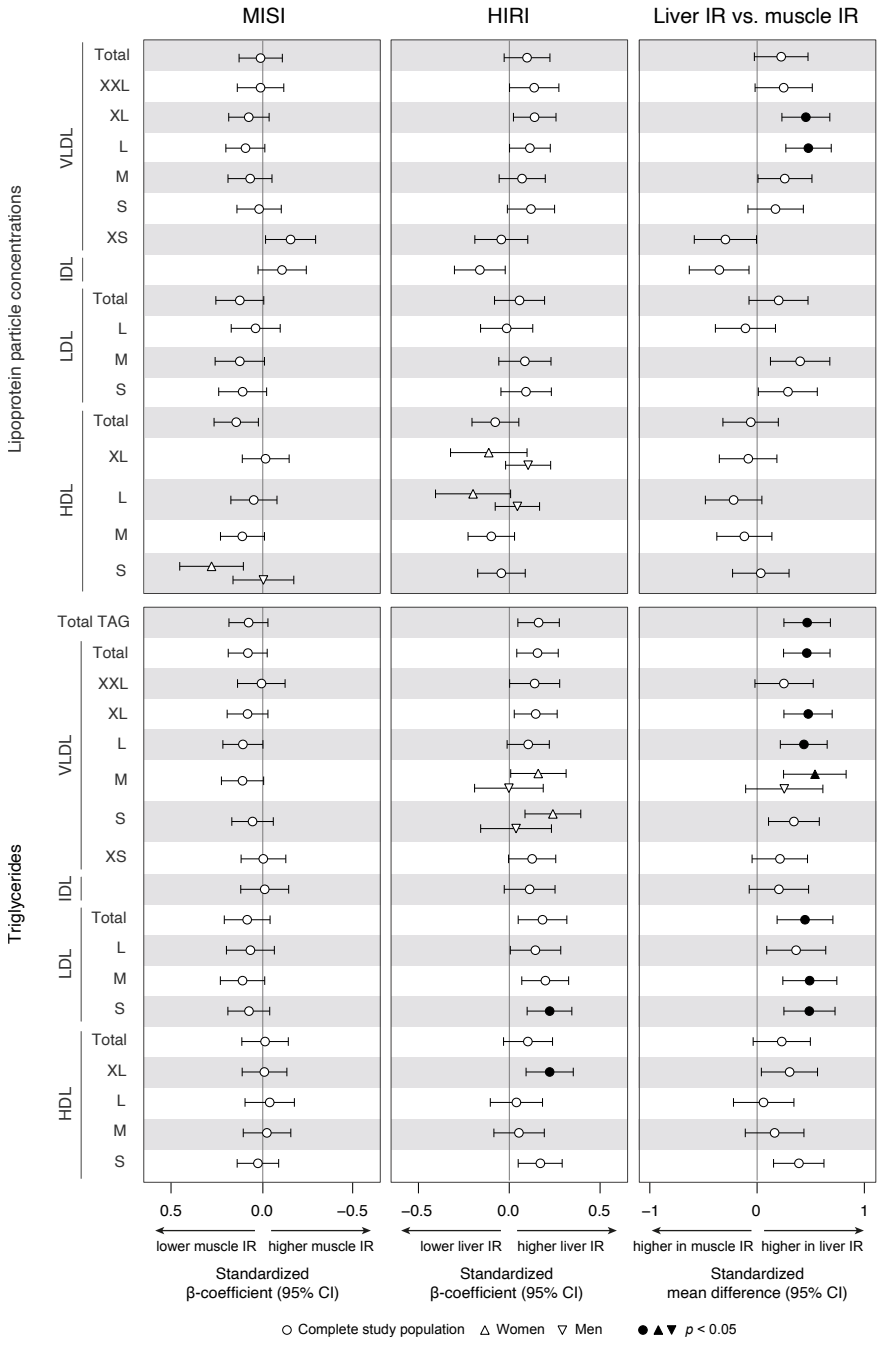

Figure 3. Postprandial (iAUC) lipoprotein particle concentrations and triglycerides (TAG) in muscle and liver IR. Left: associations of MISI with plasma metabolite iAUCs. Middle: associations of HIRI with plasma metabolite iAUCs. Right: plasma metabolite iAUCs in muscle compared to liver IR. Associations between MISI/HIRI and plasma metabolites were tested using linear regression analyses with adjustment for age, sex, centre, BMI, waist-to-hip ratio and HIRI/MISI. Differences between muscle and liver IR were tested using ANCOVA with adjustment for age, sex, centre, BMI, and waist-to-hip ratio.

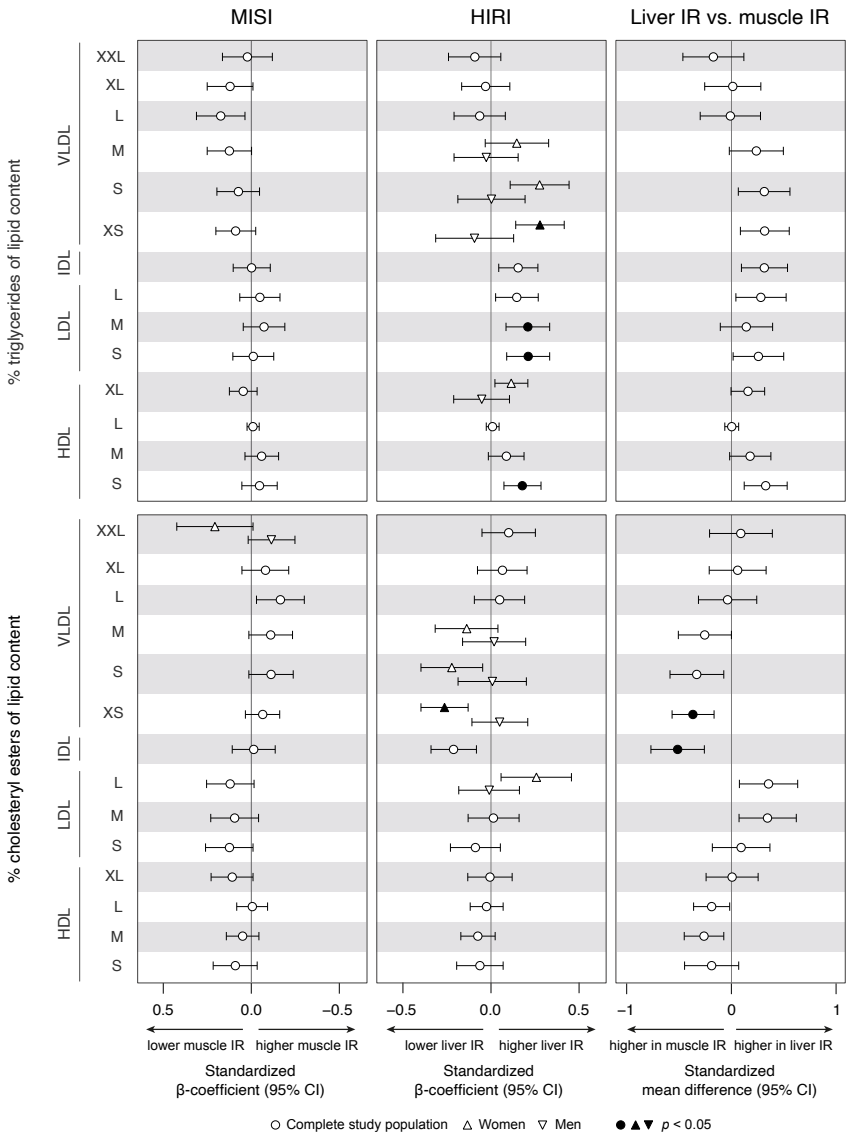

Figure 4. Postprandial (iAUC) TAG and CE content expressed as percentages of total lipoprotein lipid content in muscle and liver IR. Left: associations of MISI with plasma metabolite iAUCs. Middle: associations of HIRI with plasma metabolite iAUCs. Right: plasma metabolite iAUCs in muscle compared to liver IR. Associations between MISI/HIRI and plasma metabolites were tested using linear regression analyses with adjustment for age, sex, centre, BMI, waist-to-hip ratio and HIRI/MISI. Differences between muscle and liver IR were tested using ANCOVA with adjustment for age, sex, centre, BMI, and waist-to-hip ratio.

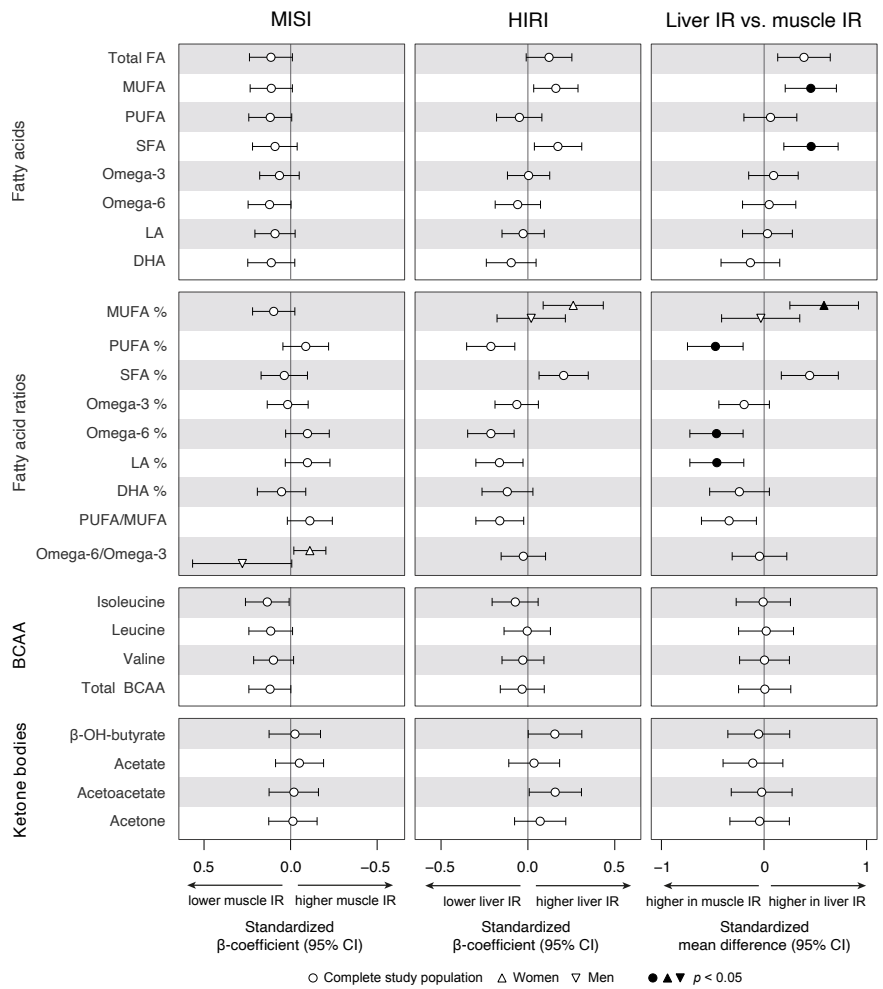

Figure 5. Postprandial (iAUC) fatty acids, fatty acid ratios, ketone bodies and branched-chain amino acids in muscle and liver IR. Left: associations of MISI with plasma metabolite iAUCs. Middle: associations of HIRI with plasma metabolite iAUCs. Right: plasma metabolite iAUCs in muscle compared to liver IR. Associations between MISI/HIRI and plasma metabolites were tested using linear regression analyses with adjustment for age, sex, centre, BMI, waist-to-hip ratio and HIRI/MISI. Differences between muscle and liver IR were tested using ANCOVA with adjustment for age, sex, centre, BMI, and waist-to-hip ratio.

Associations between MISI and HIRI with postprandial plasma metabolites

We also examined associations of MISI and HIRI with postprandial plasma metabolite iAUCs. HIRI was associated with two of the 164 absolute postprandial metabolite responses and 11 of the 82 relative metabolite measures, of which eight in women only (Fig. 2). MISI was not significantly associated to any of the postprandial metabolite responses after adjustment for multiple testing.

As for the two absolute postprandial metabolite responses, HIRI was positively associated to postprandial TAG in S LDL ($p = 0.017$) and XL HDL particles ($p = 0.034$) (Fig. 3). For the 11 relative metabolite responses, HIRI was positively associated to TAG% in S LDL particles, ($p = 0.030$), M LDL particles ($p = 0.040$), and S HDL particles ($p = 0.040$) (Fig. 4). In women only, higher HIRI was additionally associated to a higher postprandial TAG% in IDL and XS VLDL, and lower free cholesterol % in L LDL, XS and S VLDL, lower CE% in XS VLDL, and lower phospholipids % in S VLDL (Fig. 4).

Discussion

We investigated fasting and postprandial plasma metabolite profiles in tissue-specific IR. To this end, we measured 164 plasma metabolites, including lipoproteins, apolipoproteins, cholesterol, TAG, ketone bodies, and amino acids, for four hours after a high-fat mixed meal in individuals with predominant muscle IR or liver IR. Compared to individuals with muscle IR, individuals with liver IR had greater postprandial increases in concentrations of TAG in very large and large VLDL particles, small and medium LDL particles, and small HDL, while fasting lipoprotein profiles did not differ between IR phenotypes. In addition, in liver compared to muscle IR, postprandial plasma SFA and MUFA were higher, and total FA consisted of a larger percentage of SFA, and a lower percentage of PUFA.

Elevated postprandial total TAG concentrations in liver IR compared to muscle IR have been reported previously.[8] To our knowledge, this study is the first to examine circulating lipoprotein subclasses and their composition in response to a high-fat mixed meal in tissue-specific IR. Compared to muscle IR, liver IR was characterised by a larger postprandial increase in plasma TAG and cholesterol in large and very large VLDL particles, which was paralleled by increases in particle concentrations of these VLDL subclasses. This greater postprandial increase can result from higher VLDL production, reduced clearance, or both. Studies that measured VLDL kinetics using stable isotope tracers have previously shown that IR, as assessed by HOMA-IR, which mainly reflects hepatic IR, was associated with increased hepatic production of large, TAG-rich VLDL.[28,29] Hence, the greater postprandial increase in large and very large VLDL particles that we observed in people with liver IR may be due to larger hepatic VLDL production. There may be several mechanistic explanations for the greater production of large and very large VLDL particles in liver IR. Firstly, in healthy, insulin-sensitive individuals, insulin can directly inhibit VLDL production, partly by promoting the hepatic degradation of ApoB.[30-33] Hence, in liver IR, impaired insulin-mediated suppression of VLDL assembly and secretion may contribute to elevated postprandial VLDL levels, while this suppression is likely better maintained in individuals with predominant muscle IR. Secondly, insulin can increase VLDL production by inducing *de novo* lipogenesis (DNL) via activation of sterol regulatory element binding protein-1c (SREBP-1c), thereby promoting lipid synthesis.[5] This hepatic insulin action appears to be (largely) preserved in liver IR.[34] Higher hepatic IR, as measured with the gold-standard hyperinsulinemic-euglycemic clamp method, has indeed been positively associated to the relative contribution of hepatic DNL to plasma TAG in TAG-rich lipoproteins, indicating elevated DNL.[35] In the present study, individuals with liver IR had greater insulin excursions in the first hour after the meal, which may have contributed to excess VLDL production by increased DNL.

In addition to elevated postprandial VLDL-TAG in liver IR, liver IR was also characterised by higher postprandial TAG content in small and medium-sized LDL particles, and small HDL particles. As this was not paralleled by larger increases in particle concentrations, these lipoprotein particles were likely enriched in TAG in liver IR. In line with this, HIRI was positively associated to the TAG fraction of total lipid content in these LDL and HDL subclasses. A potential explanation might be enhanced transfer of TAG from large and very large VLDL particles to LDL and HDL particles by increased activity of the enzyme cholesteryl ester transfer protein (CETP). CETP facilitates the transfer of TAG from large VLDL to LDL and HDL in exchange for CE, resulting in TAG-enriched LDL and HDL.[36] CETP activity has been shown to be mainly determined by plasma TAG levels, rather than by IR,[37,38] which indicates that potentially increased CETP action in individuals with liver IR may be primarily attributable to the increased postprandial TAG in the circulation, and not to the hepatic IR itself.

In liver compared to muscle IR, the postprandial plasma FA profile was characterised by greater total SFA and MUFA concentrations, a lower percentage of PUFA of total FA, and a higher percentage of SFA of total FA 2-4 hours after mixed-meal ingestion. These results were independent of habitual dietary intake of fat, SFA, or LA, as assessed by FFQ. DNL produces mainly SFA, which can subsequently be desaturated to MUFA in the liver.[39-41] The higher early postprandial insulin response in individuals with liver IR may have promoted DNL as described above, thereby contributing to a greater postprandial increase in SFA. Recently, the proportion of SFA in VLDL has been reported to strongly correlate to the hepatic SFA fraction and, in turn, a higher hepatic SFA fraction to correlate to more severe hepatic IR.[42] Hence, higher hepatic SFA availability might also have contributed to the elevated postprandial plasma SFA in liver IR.

Hepatic IR has previously been associated with elevated fasting serum levels of various amino acids, including the branched-chain amino acids, and lower fasting serum levels of several ketone bodies, while MISI has been associated with elevated fasting levels of the ketone body acetate, and lower fasting levels of (branched-chain) amino acids isoleucine and alanine.[6] In the present study, we found similar associations between MISI and fasting plasma isoleucine and alanine, although these were no longer statistically significant after adjustment for multiple testing, and could not replicate the other associations. An important explanation for these incongruencies may be differences in the study population: we selected individuals with predominant muscle or liver IR, thus excluding insulin-sensitive individuals and individuals with combined muscle and liver IR, resulting in a smaller range of MISI and HIRI. This may also explain why we found more metabolites to significantly differ between individuals with liver or muscle IR than in the associations with MISI and HIRI. In addition, the cohorts used by Vogelzangs and colleagues[6] were much larger (n=634 and n=540) than our study population.

It is well established that sex differences in lipid metabolism exist, which may contribute to differences in the aetiology of chronic cardiometabolic diseases between men and women.[43] Interestingly, we also observed sex differences, with a more pronounced link between liver IR and postprandial lipid profile in women. Women generally have a more favourable plasma lipid profile than men, but various studies indicate that in impaired metabolic health, i.e. obesity or T2DM, women show greater abnormalities in lipid and lipoprotein metabolism than men.[44-49] Similarly, hepatic IR has previously been associated with plasma lipid abnormalities in the fasting state - including elevated TAG, DAG, and BCAA - in women, and not in men.[6,7] Lipid metabolism in women is also affected by sex hormones and menopausal state.[43,50] The majority of women in our study – 84% - was postmenopausal, and the limited number of premenopausal women (n=21) did not allow for performing stratified analyses. The observed sex differences in the relationship between tissue-specific IR and postprandial lipoprotein profile highlight the importance of taking sexual dimorphism into account and warrant further research to elucidate underlying mechanisms.

The lipoprotein profile we observed in liver compared to muscle IR – elevated postprandial TAG in larger VLDL and smaller LDL and HDL particles – is common in IR and T2DM.[31,32,51,52] Such a lipid profile is considered to be highly atherogenic and has consistently been associated with increased CVD risk.[9-11,53,54] Our findings show that this postprandial lipid profile is specifically related to liver IR, and less so to muscle IR, despite similar body fat percentage, liver fat, and whole-body insulin sensitivity. Most individuals spend the majority of the day in the postprandial state. Even in healthy, insulin-sensitive individuals, plasma TAG concentrations progressively rise throughout the day upon repeated meal consumption, returning only to fasting

levels during sleep.[55] Thus, individuals with liver IR may be at increased risk of developing cardiometabolic disease, compared to individuals with muscle IR.

Interestingly, the more dyslipidemic lipoprotein profile in liver compared to muscle IR was not observed in the fasting state. Differences between IR phenotypes only became apparent after challenging the body with a high-fat mixed meal. In both individuals with liver and muscle IR, the majority had fasting TAG concentrations in the normal range (<1.7 mmol/L): 71% in muscle IR and 64% in liver IR. In addition, a large majority was normoglycemic: 77% in muscle IR and 78% in liver IR. These findings thus indicate that liver IR in particular, is accompanied by early perturbations in postprandial lipid metabolism that are not evident in the fasting state yet compared to muscle IR. Detection of metabolic perturbations at this early stage - before the onset of overt metabolic disease - provides an opportunity for timely prevention of progression to cardiometabolic disease by lifestyle interventions such as dietary modification, exercise, and weight loss.

A major strength of this study is the extensive metabolic profiling of tissue-specific IR in both the fasting and the postprandial state, thereby broadening and deepening the characterisation of plasma lipid profiles in tissue-specific IR and providing more insights into the metabolic abnormalities that are related to muscle or liver IR. It is as of yet unknown whether dysregulated lipid metabolism is a cause or consequence of hepatic IR. Due to the cross-sectional design of this study, we cannot make any causal inferences about the nature of the observed relationship between tissue-specific IR and postprandial plasma lipid profiles. Another limitation of this study is the use of OGTT-derived measures for the assessment of tissue-specific IR. Postprandial glycemic and insulin responses are also affected by gastro-intestinal factors such as gastric emptying and the incretin response.[56] Therefore, MISI and HIRI provide a less precise estimation of tissue-specific IR compared to the gold-standard two-step hyperinsulinemic-euglycemic clamp. Nevertheless, these indices have been validated against the gold-standard clamp[16] and we have previously shown that using these OGTT-derived measures, we could identify distinct metabolic phenotypes in various cohorts.[6,7,57] In addition, our study population consisted only of individuals with some degree of tissue-specific IR. Therefore, we cannot conclude anything on postprandial metabolic profiles in tissue-specific IR compared to healthy, insulin-sensitive controls. Finally, we sampled blood until four hours after consumption of the meal, at which many plasma lipids are at their peak. Future studies should employ longer sampling times until 6-8 hours post-meal to allow for examination of the (rate of) return to fasting levels or the effects of a second meal.

Conclusion

In conclusion, individuals with liver IR or muscle IR have distinct postprandial plasma lipoprotein responses after a mixed meal, despite similar fasting lipoprotein profiles, body fat percentage, liver fat, and whole-body insulin sensitivity. Liver IR was characterised by a more dyslipidemic postprandial profile, reflected by elevated TAG in the larger VLDL and the smaller LDL and HDL subclasses, which points towards more impaired hepatic lipid metabolism in liver compared to muscle IR. Therefore, improving postprandial lipid metabolism with lifestyle modifications to prevent the development of cardiometabolic disease may be particularly important for individuals with predominant liver IR.

Acknowledgements

We would like to sincerely thank our study participants for their commitment. In addition, we thank W. Sluijsmans, H. Aydeniz, Y. Essers, and N. Hoebers for their support in performing lab analyses and E. Siebelink, H. Fick, K. Manusama, M. Grootte Bromhaar, O. Paling, M. Ummels, A. Hoge, S. Bowser, L. Omary, and all students for their invaluable contribution to the execution of the study.

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Supplementary material

These tables only include metabolite subclasses that were significantly different between muscle IR and liver IR or significantly associated with MISI or HIRI. Full supplemental tables can be found online: https://doi.org/10.4121/4c08e194-9715-4f5c-8515-55df53d2a3eb.

Table S2a. Postprandial plasma metabolites (iAUC) in muscle (muscle IR) and liver insulin resistance (liver IR)

	muscle IR			liver IR			Liver IR vs. muscle IR					
							Std. mean difference			P-values		
	Mean	95% CI		Mean	95% CI		Mean	95% CI		Crude P	Adjusted P	Sex interaction
Triglycerides												
Total TAG, mmol/L	13.0	11.4	14.6	18.7	16.4	21.1	0.47	0.68	0.25	<0.001	**0.001**	0.15
VLDL TAG, mmol/L	11.7	10.3	13.1	16.9	14.7	19.1	0.46	0.68	0.25	<0.001	**0.001**	0.15
LDL TAG, mmol/L	0.37	0.27	0.46	0.65	0.53	0.76	0.45	0.71	0.18	<0.001	**0.035**	0.22
HDL TAG, mmol/L	0.86	0.75	0.97	1.03	0.86	1.20	0.23	0.50	-0.04	0.09	1.00	0.89
Total lipids												
Total lipids, mmol/L	16.6	12.4	20.7	24.5	19.4	29.6	0.22	0.47	-0.04	0.10	1.00	0.27
Lipids in VLDL, mmol/L	14.3	12.3	16.3	20.8	17.9	23.7	0.41	0.63	0.19	<0.001	**0.012**	0.17
Lipids in LDL - women, mmol/L	-1.99	-4.05	0.06	2.36	-0.14	4.87	0.38	0.75	0.02	0.04	1.00	**0.05**
Lipids in LDL - men, mmol/L	0.38	-1.66	2.42	0.64	-1.66	2.93	-0.08	0.29	-0.44	0.68	1.00	
Lipids in HDL, mmol/L	3.0	1.9	4.1	2.5	-0.1	5.1	-0.11	0.15	-0.36	0.42	1.00	0.50
Other lipids												
Phosphoglycerides (PG), mmol/L	4.4	3.4	5.4	5.0	3.4	6.5	0.00	0.27	-0.26	0.98	1.00	1.00
TAG/PG, ratio	4.0	3.5	4.5	5.8	5.0	6.5	0.38	0.56	0.19	<0.001	**0.003**	0.14
Cholines, mmol/L	3.0	1.9	4.0	3.5	1.9	5.1	-0.02	0.24	-0.28	0.88	1.00	0.87
Phosphatidylcholines, mmol/L	4.2	3.3	5.0	4.5	3.0	6.0	-0.05	0.21	-0.31	0.72	1.00	0.86
Sphingomyelins, mmol/L	-0.35	-0.57	-0.13	-0.25	-0.54	0.03	-0.01	0.26	-0.27	0.97	1.00	0.24
Fatty acids												
Total FA, mmol/L	51.7	44.8	58.6	71.1	61.2	81.0	0.39	0.65	0.14	<0.001	0.11	0.26
Omega-3, mmol/L	1.9	1.5	2.2	2.3	1.8	2.9	0.10	0.34	-0.14	0.43	1.00	0.93
Omega-6, mmol/L	4.5	2.7	6.3	6.5	4.2	8.9	0.05	0.31	-0.21	0.69	1.00	0.39
PUFA, mmol/L	6.4	4.3	8.4	8.9	6.1	11.6	0.06	0.32	-0.19	0.62	1.00	0.45
MUFA, mmol/L	18.7	16.3	21.2	26.7	23.1	30.3	0.46	0.71	0.21	<0.001	**0.012**	0.11
SFA, mmol/L	26.6	23.7	29.5	35.5	30.9	40.1	0.46	0.73	0.20	<0.001	**0.028**	0.46
Linoleic acid, mmol/L	4.8	2.9	6.6	7.0	4.7	9.3	0.04	0.28	-0.20	0.76	1.00	0.25
DHA, mmol/L	0.01	-0.19	0.20	-0.02	-0.33	0.28	-0.13	0.15	-0.42	0.37	1.00	0.72
Fatty acid ratios												
Omega 3 %	1.1	-0.6	2.8	0.1	-2.5	2.8	-0.19	0.05	-0.44	0.12	1.00	0.28
Omega 6 %	-96.8	-105.0	-88.6	-119.5	-132.7	-106.4	-0.46	-0.20	-0.72	<0.001	**0.021**	0.27
PUFA %	-95.7	-104.6	-86.8	-119.4	-134.2	-104.6	-0.47	-0.20	-0.74	<0.001	**0.025**	0.23
MUFA % - women	34.7	27.1	42.3	56.4	46.1	66.7	0.59	0.92	0.25	<0.001	**0.027**	**0.01**
MUFA % - men	37.5	28.9	46.1	36.2	27.0	45.4	-0.03	0.35	-0.41	0.87	1.00	
SFA %	60.0	54.8	65.1	71.8	62.3	81.4	0.45	0.72	0.17	<0.001	0.07	0.75
LA %	-67.5	-75.2	-59.8	-83.6	-96.4	-70.7	-0.46	-0.20	-0.72	<0.001	**0.027**	0.82
DHA %	-4.9	-6.3	-3.5	-6.2	-8.4	-4.1	-0.24	0.05	-0.53	0.11	1.00	0.37
PUFA/MUFA, ratio	-5.8	-6.5	-5.1	-7.0	-7.9	-6.1	-0.34	-0.07	-0.61	0.01	0.48	0.06
Omega 6/Omega 3, ratio	-30.3	-45.6	-15.0	-39.3	-51.1	-27.5	-0.04	0.22	-0.31	0.74	1.00	0.47
Lipoprotein subclasses												
Very large VLDL (average diameter 64.0 nm)												
XL VLDL particles, µmol/l	0.05	0.05	0.06	0.08	0.07	0.09	0.45	0.67	0.23	<0.001	**0.004**	0.24
Lipids in XL VLDL, mmol/L	3.29	2.90	3.67	4.67	4.05	5.29	0.46	0.68	0.23	<0.001	**0.003**	0.25
Phospholipids in XL VLDL, mmol/L	0.59	0.52	0.66	0.80	0.69	0.91	0.38	0.62	0.14	<0.001	0.06	0.46

Table S2a. Continued

	muscle IR			liver IR			Liver IR vs. muscle IR					
							Std. mean difference			P-values		
	Mean	95% CI		Mean	95% CI		Mean	95% CI		Crude P	Adjusted P	Sex interaction
Very large VLDL (average diameter 64.0 nm)												
Cholesterol in XL VLDL, mmol/L	0.37	0.31	0.43	0.57	0.48	0.65	0.40	0.63	0.18	<0.001	**0.019**	0.19
Cholesteryl esters in XL VLDL, mmol/L	0.09	0.07	0.12	0.18	0.15	0.22	0.41	0.64	0.18	<0.001	**0.020**	0.05
Free cholesterol in XL VLDL, mmol/L	0.28	0.24	0.31	0.38	0.32	0.44	0.36	0.60	0.12	<0.001	0.12	0.50
TAG in XL VLDL, mmol/L	2.33	2.06	2.59	3.30	2.87	3.73	0.47	0.70	0.25	<0.001	**0.002**	0.24
Large VLDL (average diameter 53.6 nm)												
L VLDL particles, µmol/l	0.10	0.08	0.12	0.16	0.14	0.18	0.48	0.69	0.26	<0.001	**0.001**	0.06
Lipids in L VLDL, mmol/L	3.16	2.59	3.74	5.04	4.29	5.79	0.43	0.65	0.22	<0.001	**0.004**	0.08
Phospholipids in L VLDL, mmol/L	0.65	0.54	0.76	1.02	0.88	1.17	0.44	0.65	0.23	<0.001	**0.002**	0.07
Cholesterol in L VLDL, mmol/L	0.42	0.29	0.55	0.79	0.63	0.94	0.39	0.60	0.17	<0.001	**0.023**	0.06
Cholesteryl esters in L VLDL, mmol/L	0.08	0.02	0.15	0.22	0.15	0.30	0.28	0.52	0.05	0.02	0.66	0.10
Free cholesterol in L VLDL, mmol/L	0.33	0.27	0.40	0.56	0.47	0.65	0.45	0.66	0.24	<0.001	**0.002**	0.06
TAG in L VLDL, mmol/L	2.09	1.74	2.45	3.23	2.76	3.69	0.43	0.65	0.22	<0.001	**0.005**	0.10
Medium VLDL (average diameter 44.5 nm)												
M VLDL particles, µmol/l	0.01	-0.02	0.05	0.08	0.04	0.12	0.26	0.51	0.01	0.04	1.00	0.05
Lipids in M VLDL, mmol/L	1.04	0.40	1.69	2.61	1.82	3.39	0.32	0.56	0.08	0.01	0.37	0.05
Phospholipids in M VLDL, mmol/L	0.09	-0.02	0.20	0.29	0.15	0.43	0.20	0.46	-0.05	0.12	1.00	0.08
Cholesterol in M VLDL, mmol/L	-0.50	-0.62	-0.37	-0.53	-0.72	-0.35	-0.17	0.12	-0.45	0.25	1.00	0.57
Cholesteryl esters in M VLDL, mmol/L	-0.38	-0.46	-0.30	-0.49	-0.60	-0.38	-0.30	-0.03	-0.58	0.03	1.00	0.97
Free cholesterol in M VLDL, mmol/L	-0.11	-0.18	-0.05	-0.05	-0.14	0.04	0.05	0.33	-0.22	0.70	1.00	0.22
TAG in M VLDL - women, mmol/L	1.57	0.89	2.26	3.54	2.75	4.33	0.54	0.83	0.25	<0.001	**0.015**	**0.05**
TAG in M VLDL - men, mmol/L	1.22	0.53	1.91	1.95	1.15	2.75	0.25	0.61	-0.11	0.17	1.00	
Medium LDL (average diameter 23.0 nm)												
M LDL particles, mmol/L	0.45	0.15	0.76	1.24	0.78	1.69	0.40	0.68	0.12	0.01	0.19	0.11
Lipids in M LDL, mmol/L - women	0.17	-0.52	0.86	2.15	1.29	3.01	0.61	0.97	0.24	<0.001	**0.048**	**0.02**
Lipids in M LDL, mmol/L - men	0.85	0.12	1.57	1.15	0.39	1.90	0.04	0.42	-0.34	0.83	1.00	
Phospholipids in M LDL, mmol/L - women	-0.12	-0.27	0.04	0.23	0.04	0.42	0.42	0.79	0.05	0.03	1.00	**0.03**
Phospholipids in M LDL, mmol/L - men	0.07	-0.08	0.22	0.08	-0.10	0.26	-0.12	0.25	-0.48	0.53	1.00	
Cholesterol in M LDL, mmol/L - women	0.17	-0.36	0.69	1.69	1.01	2.38	0.62	0.99	0.26	<0.001	**0.039**	**0.02**
Cholesterol in M LDL, mmol/L - men	0.67	0.10	1.23	0.91	0.35	1.48	0.07	0.45	-0.32	0.72	1.00	
Cholesteryl esters in M LDL, mmol/L - women	0.48	0.04	0.93	1.86	1.25	2.47	0.67	1.03	0.30	<0.001	**0.016**	**0.03**
Cholesteryl esters in M LDL, mmol/L - men	0.79	0.31	1.27	1.09	0.60	1.59	0.14	0.52	-0.24	0.47	1.00	
Free cholesterol in M LDL, mmol/L	-0.25	-0.33	-0.17	-0.17	-0.28	-0.07	0.03	0.29	-0.24	0.85	1.00	0.09
TAG in M LDL, mmol/L	0.11	0.09	0.14	0.20	0.17	0.23	0.49	0.74	0.24	<0.001	**0.006**	0.13
Small LDL (average diameter 18.7 nm)												
S LDL particles, mmol/L	0.09	0.01	0.17	0.27	0.17	0.37	0.28	0.56	0.01	0.04	1.00	0.25
Lipids in S LDL, mmol/L - women	0.04	-0.17	0.25	0.70	0.42	0.99	0.60	0.96	0.24	<0.001	**0.046**	**0.02**
Lipids in S LDL, mmol/L - men	0.31	0.08	0.53	0.42	0.15	0.69	0.01	0.39	-0.37	0.96	1.00	
Phospholipids in S LDL, mmol/L - women	-0.04	-0.10	0.02	0.14	0.06	0.23	0.57	0.95	0.20	<0.001	0.11	**0.02**
Phospholipids in S LDL, mmol/L - men	0.04	-0.02	0.10	0.07	-0.01	0.14	-0.03	0.35	-0.40	0.89	1.00	
Cholesterol in S LDL, mmol/L - women	-0.05	-0.20	0.09	0.36	0.17	0.55	0.55	0.91	0.19	<0.001	0.13	**0.02**
Cholesterol in S LDL, mmol/L - men	0.15	-0.01	0.30	0.20	0.03	0.38	-0.02	0.36	-0.40	0.91	1.00	
Cholesteryl esters in S LDL, mmol/L - women	0.06	-0.05	0.17	0.39	0.25	0.53	0.60	0.95	0.25	<0.001	**0.035**	**0.04**
Cholesteryl esters in S LDL, mmol/L - men	0.19	0.07	0.30	0.27	0.13	0.40	0.08	0.46	-0.31	0.70	1.00	
Free cholesterol in S LDL, mmol/L - women	-0.12	-0.16	-0.07	-0.04	-0.11	0.03	0.29	0.68	-0.09	0.14	1.00	**0.05**
Free cholesterol in S LDL, mmol/L - men	-0.04	-0.09	0.01	-0.06	-0.12	-0.01	-0.23	0.14	-0.59	0.22	1.00	
TAG in S LDL, mmol/L	0.13	0.11	0.14	0.18	0.16	0.21	0.49	0.72	0.25	<0.001	**0.003**	0.26

Table S2a. Continued

	muscle IR			liver IR			Liver IR vs. muscle IR					
							Std. mean difference			P-values		
	Mean	95% CI		Mean	95% CI		Mean	95% CI		Crude P	Adjusted P	Sex interaction
Relative lipoprotein lipid concentrations												
Very small VLDL (average diameter 31.3 nm)												
Phospholipids% in XS VLDL	-10.4	-15.6	-5.2	-13.8	-19.5	-8.0	-0.08	0.21	-0.36	0.59	1.00	0.15
Cholesterol% in XS VLDL	-31.6	-41.6	-21.5	-56.7	-68.4	-45.0	-0.34	-0.12	-0.55	<0.001	0.085	0.18
Cholesteryl esters% in XS VLDL	-13.1	-21.5	-4.8	-35.5	-45.2	-25.8	-0.34	-0.14	-0.54	<0.001	**0.036**	0.26
Free cholesterol% in XS VLDL	-18.4	-21.0	-15.8	-21.2	-25.1	-17.4	-0.15	0.13	-0.43	0.30	1.00	0.18
TAG% in XS VLDL	42.0	29.4	54.6	70.5	56.7	84.3	0.33	0.57	0.10	0.01	0.233	0.09
IDL (average diameter 28.6 nm)												
Phospholipids% in IDL	-16.3	-20.0	-12.7	-11.3	-16.4	-6.2	0.29	0.54	0.04	0.02	0.897	0.46
Cholesterol% in IDL	10.0	3.5	16.5	-5.0	-13.0	3.0	-0.41	-0.17	-0.66	<0.001	**0.044**	0.68
Cholesteryl esters% in IDL	32.7	26.2	39.3	14.9	5.2	24.7	-0.49	-0.23	-0.74	<0.001	**0.008**	0.56
Free cholesterol% in IDL	-22.7	-26.9	-18.6	-20.0	-25.8	-14.1	0.15	0.42	-0.12	0.27	1.00	0.75
TAG% in IDL	6.3	1.7	11.0	16.3	11.3	21.4	0.33	0.55	0.11	<0.001	0.144	0.99
Large LDL (average diameter 25.5 nm)												
Phospholipids% in L LDL	3.5	0.3	6.7	-5.0	-8.4	-1.7	-0.40	-0.13	-0.68	<0.001	0.157	0.11
Cholesterol% in L LDL	-15.8	-19.7	-11.9	-11.5	-16.7	-6.2	0.08	0.34	-0.19	0.57	1.00	0.12
Cholesteryl esters% in L LDL	-4.4	-9.4	0.6	8.8	2.7	14.9	0.38	0.66	0.10	0.01	0.280	0.06
Free cholesterol% in L LDL	-11.3	-14.0	-8.7	-20.3	-24.2	-16.4	-0.47	-0.23	-0.71	<0.001	**0.006**	0.26
TAG% in L LDL	12.2	9.7	14.8	16.5	13.0	20.0	0.29	0.54	0.05	0.02	0.669	0.64

Values are geometric means with 95% confidence intervals. Differences between muscle and liver IR were tested using ANCOVA with adjustment for age, sex, centre, BMI, and waist-to-hip ratio.

Table S2b. Associations between HIRI and postprandial plasma metabolites (iAUC)

	HIRI					
	Std. β			P-values		
	Mean	95% CI		Crude P	Adjusted P	Sex interaction
Other lipids						
Phosphoglycerides (PG), mmol/L	-0.06	-0.19	0.08	0.40	1.00	0.39
TAG/PG, ratio - women	0.23	0.10	0.36	<0.001	**0.020**	**0.036**
TAG/PG, ratio - men	0.04	-0.10	0.18	0.58	1.00	
Cholines, mmol/L	-0.07	-0.20	0.07	0.33	1.00	0.46
Phosphatidylcholines, mmol/L	-0.07	-0.20	0.06	0.27	1.00	0.47
Sphingomyelins, mmol/L	-0.05	-0.18	0.08	0.44	1.00	0.85
Lipoprotein subclasses						
Small LDL (average diameter 18.7 nm)						
S LDL particles, mmol/L	0.09	-0.05	0.23	0.19	1.00	0.75
Lipids in S LDL, mmol/L	0.10	-0.03	0.23	0.14	1.00	0.07
Phospholipids in S LDL, mmol/L - women	0.21	0.03	0.40	0.03	1.00	**0.026**
Phospholipids in S LDL, mmol/L - men	-0.02	-0.21	0.18	0.87	1.00	
Cholesterol in S LDL, mmol/L	0.06	-0.08	0.19	0.39	1.00	0.13
Cholesteryl esters in S LDL, mmol/L	0.07	-0.07	0.20	0.33	1.00	0.18
Free cholesterol in S LDL, mmol/L	0.03	-0.11	0.17	0.68	1.00	0.12
TAG in S LDL, mmol/L	0.22	0.10	0.34	<0.001	**0.017**	0.29
Very large HDL (average diameter 14.3 nm)						
XL HDL particles, mmol/L - women	-0.11	-0.32	0.10	0.29	1.00	**0.028**
XL HDL particles, mmol/L - men	0.10	-0.02	0.23	0.10	1.00	
Lipids in XL HDL, mmol/L - women	-0.18	-0.38	0.02	0.08	1.00	**0.007**
Lipids in XL HDL, mmol/L - men	0.09	-0.04	0.22	0.17	1.00	
Phospholipids in XL HDL, mmol/L - women	-0.20	-0.40	0.01	0.06	1.00	**0.008**
Phospholipids in XL HDL, mmol/L - men	0.08	-0.05	0.21	0.23	1.00	
Cholesterol in XL HDL, mmol/L - women	-0.19	-0.37	-0.01	0.04	1.00	**0.005**
Cholesterol in XL HDL, mmol/L - men	0.07	-0.07	0.21	0.29	1.00	
Cholesteryl esters in XL HDL, mmol/L - women	-0.19	-0.37	0.00	0.05	1.00	**0.012**
Cholesteryl esters in XL HDL, mmol/L - men	0.05	-0.10	0.20	0.49	1.00	
Free cholesterol in XL HDL, mmol/L - women	-0.14	-0.33	0.04	0.12	1.00	**0.007**
Free cholesterol in XL HDL, mmol/L - men	0.12	-0.05	0.29	0.16	1.00	
TAG in XL HDL, mmol/L	0.22	0.09	0.35	<0.001	**0.034**	0.58

Table S2b. Continued

	HIRI					
	Std. β			P-values		
	Mean	95% CI		Crude P	Adjusted P	Sex interaction
Relative lipoprotein lipid concentrations						
Small VLDL (average diameter 36.8 nm)						
Phospholipids% in S VLDL - women	-0.31	-0.48	-0.14	<0.001	**0.015**	**0.007**
Phospholipids% in S VLDL - men	-0.02	-0.20	0.16	0.82	1.00	
Cholesterol% in S VLDL - women	-0.26	-0.43	-0.09	<0.001	0.11	**0.006**
Cholesterol% in S VLDL - men	0.00	-0.19	0.20	0.98	1.00	
Cholesteryl esters% in S VLDL - women	-0.22	-0.40	-0.05	0.01	0.48	**0.012**
Cholesteryl esters% in S VLDL - men	0.01	-0.19	0.20	0.94	1.00	
Free cholesterol% in S VLDL - women	-0.32	-0.47	-0.16	<0.001	**0.006**	**0.003**
Free cholesterol% in S VLDL - men	-0.01	-0.20	0.19	0.96	1.00	
TAG% in S VLDL - women	0.28	0.11	0.44	<0.001	0.05	**0.005**
TAG% in S VLDL - men	0.00	-0.19	0.19	0.98	1.00	
Very small VLDL (average diameter 31.3 nm)						
Phospholipids% in XS VLDL	0.03	-0.12	0.17	0.70	1.00	0.25
Cholesterol% in XS VLDL - women	-0.297	-0.435	-0.159	<0.001	**0.002**	**0.000**
Cholesterol% in XS VLDL - men	0.068	-0.111	0.247	0.45	1.00	
Cholesteryl esters% in XS VLDL - women	-0.263	-0.397	-0.129	<0.001	**0.006**	**0.001**
Cholesteryl esters% in XS VLDL - men	0.050	-0.108	0.208	0.53	1.00	
Free cholesterol% in XS VLDL - women	-0.282	-0.442	-0.123	<0.001	**0.024**	**0.012**
Free cholesterol% in XS VLDL - men	0.108	-0.167	0.383	0.44	1.00	
TAG% in XS VLDL - women	0.277	0.140	0.414	<0.001	**0.004**	**0.001**
TAG% in XS VLDL - men	-0.094	-0.315	0.127	0.40	1.00	
Large LDL (average diameter 25.5 nm)						
Phospholipids% in L LDL - women	-0.266	-0.456	-0.076	0.01	0.24	**0.001**
Phospholipids% in L LDL - men	0.010	-0.169	0.189	0.91	1.00	
Cholesterol% in L LDL	-0.01	-0.14	0.13	0.92	1.00	0.09
Cholesteryl esters% in L LDL - women	0.26	0.06	0.46	0.01	0.46	**0.003**
Cholesteryl esters% in L LDL - men	-0.01	-0.18	0.16	0.91	1.00	
Free cholesterol% in L LDL - women	-0.33	-0.50	-0.17	<0.001	**0.005**	**0.005**
Free cholesterol% in L LDL - men	-0.04	-0.23	0.15	0.68	1.000	
TAG% in L LDL	0.15	0.03	0.27	0.02	0.68	0.30

Table S2b. Continued

	HIRI					
	Std. β			P-values		
	Mean	95% CI		Crude P	Adjusted P	Sex interaction
Medium LDL (average diameter 23.0 nm)						
Phospholipids% in M LDL	-0.02	-0.16	0.13	0.81	1.00	0.94
Cholesterol% in M LDL	-0.05	-0.20	0.09	0.48	1.00	0.67
Cholesteryl esters% in M LDL	0.01	-0.13	0.16	0.85	1.00	0.80
Free cholesterol% in M LDL	-0.08	-0.22	0.05	0.23	1.00	0.32
TAG% in M LDL	0.21	0.09	0.33	<0.001	**0.040**	0.21
Small LDL (average diameter 18.7 nm)						
Phospholipids% in S LDL	0.04	-0.10	0.19	0.54	1.00	0.55
Cholesterol% in S LDL	-0.20	-0.34	-0.07	<0.001	0.15	0.16
Cholesteryl esters% in S LDL	-0.09	-0.23	0.05	0.22	1.00	0.35
Free cholesterol% in S LDL	-0.08	-0.22	0.05	0.22	1.00	0.89
TAG% in S LDL	0.21	0.09	0.33	<0.001	**0.030**	0.22
Small HDL (average diameter 8.7 nm)						
Phospholipids% in S HDL - women	-0.18	-0.39	0.04	0.10	1.00	**0.046**
Phospholipids% in S HDL - men	0.08	-0.10	0.26	0.39	1.00	
Cholesterol% in S HDL	-0.07	-0.20	0.07	0.34	1.00	0.43
Cholesteryl esters% in S HDL	-0.06	-0.19	0.07	0.34	1.00	0.75
Free cholesterol% in S HDL	-0.03	-0.17	0.11	0.70	1.00	0.07
TAG% in S HDL	0.18	0.07	0.28	<0.001	**0.040**	0.18

Associations between HIRI and plasma metabolite iAUCs were tested using linear regression analyses with adjustment for age, sex, centre, BMI, waist-to-hip ratio and HIRI/MISI.

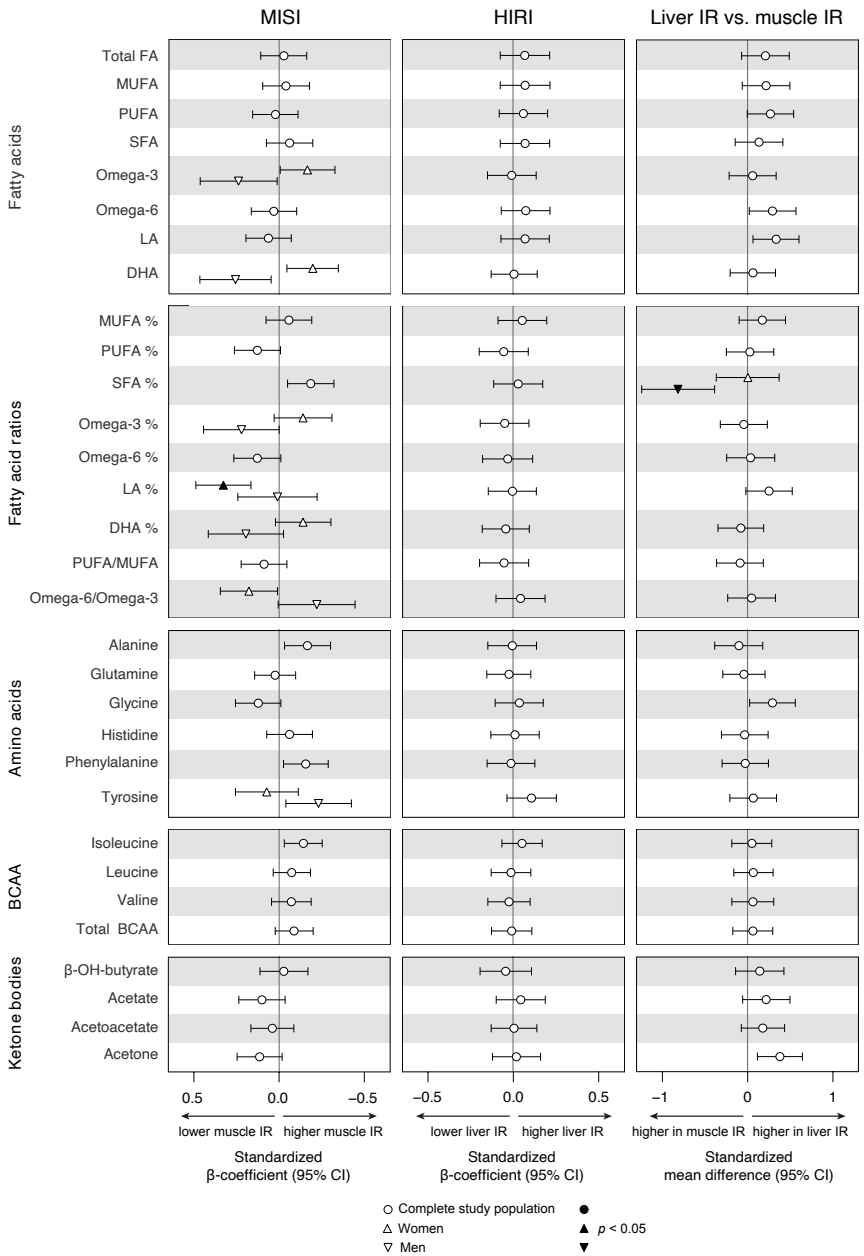

Figure S1. Fasting plasma fatty acids, fatty acid ratios, (branched-chain) amino acids, and ketone bodies in muscle and liver IR. Left: associations of MISI with fasting plasma metabolites. Middle: associations of HIRI with fasting plasma metabolites. Right: fasting plasma metabolite

in muscle compared to liver IR. Associations between MISI/HIRI and plasma metabolites were tested using linear regression analyses with adjustment for age, sex, center, BMI, waist-to-hip ratio and HIRI/MISI. Differences between muscle and liver IR were tested using ANCOVA with adjustment for age, sex, center, BMI, and waist-to-hip ratio.

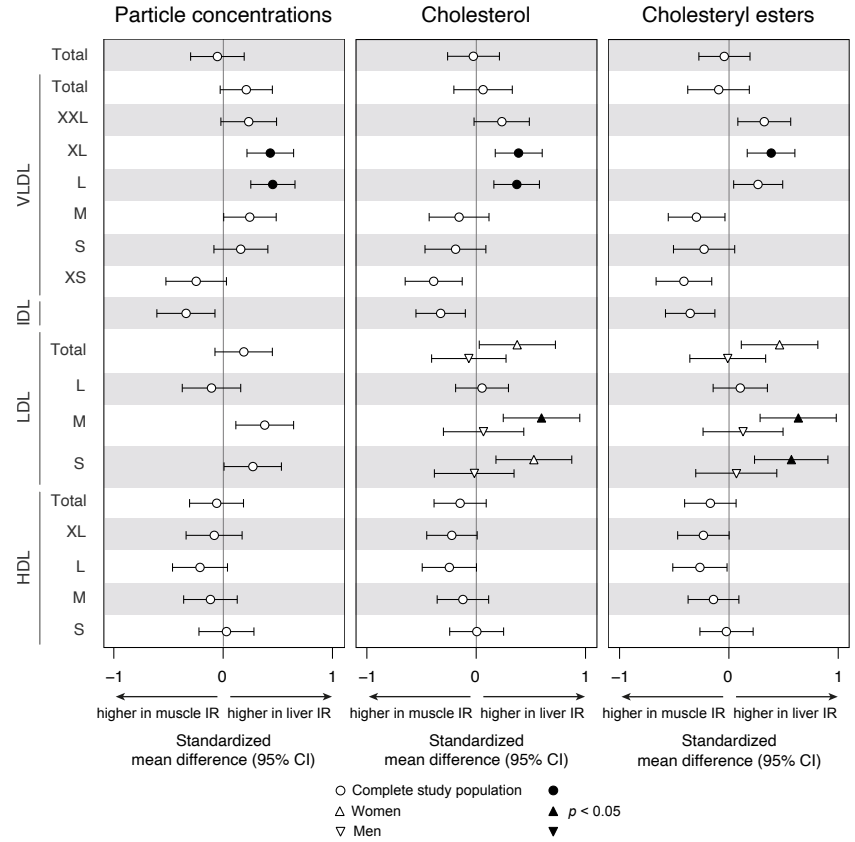

Figure S2. Postprandial (iAUC) lipoprotein particle concentrations, cholesterol, and cholesteryl esters in liver IR versus muscle IR. Differences between muscle and liver IR were tested using ANCOVA with adjustment for age, sex, center, BMI, and waist-to-hip ratio.

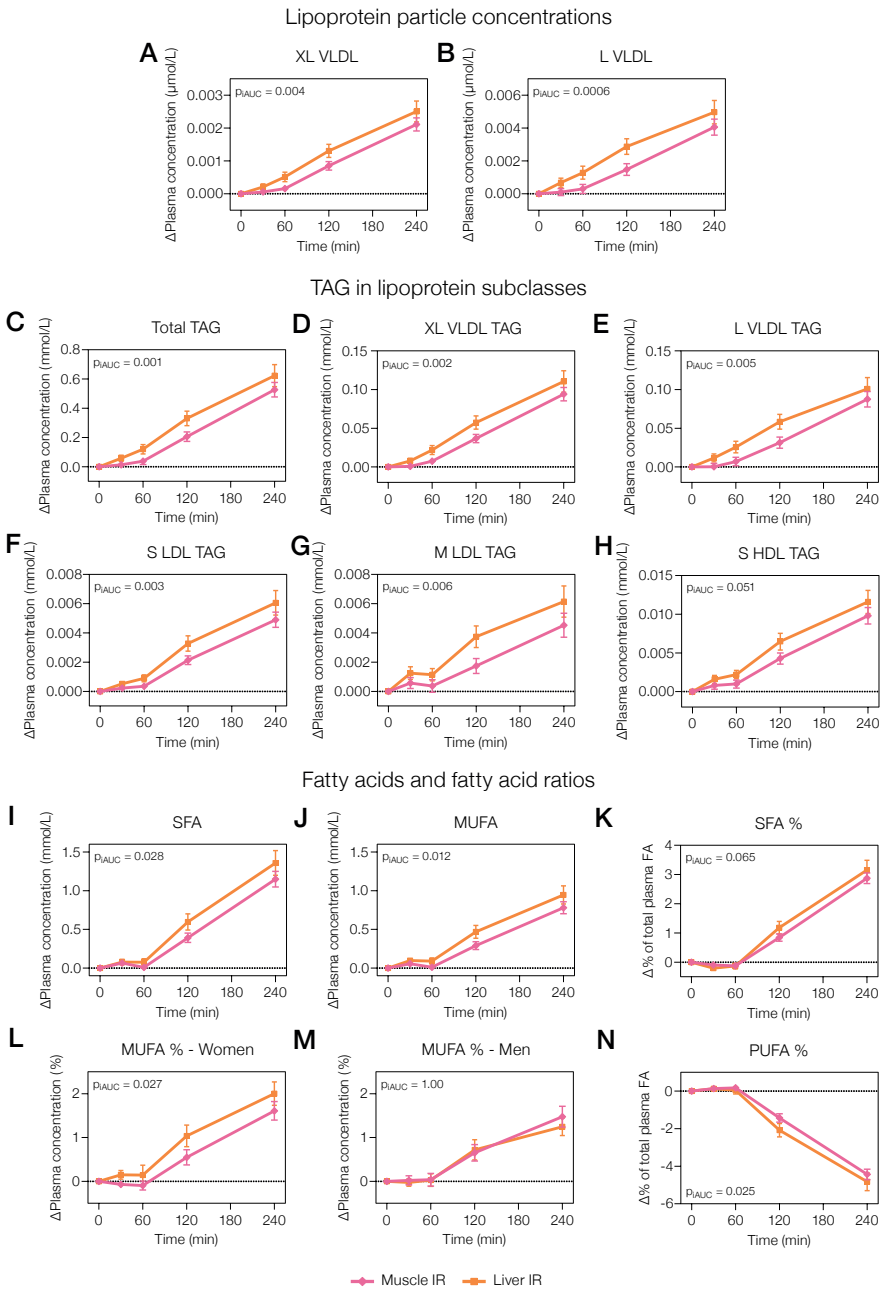

Figure S3. Plasma metabolite responses to a high-fat mixed meal. Responses were defined as change from fasting (value at postprandial timepoint – fasting value) and data are shown as means with 95% confidence intervals. Differences in incremental area under the curves (iAUCs) between liver IR and muscle IR were tested using ANCOVA with adjustment for age, sex, center, BMI, and waist-to-hip ratio.

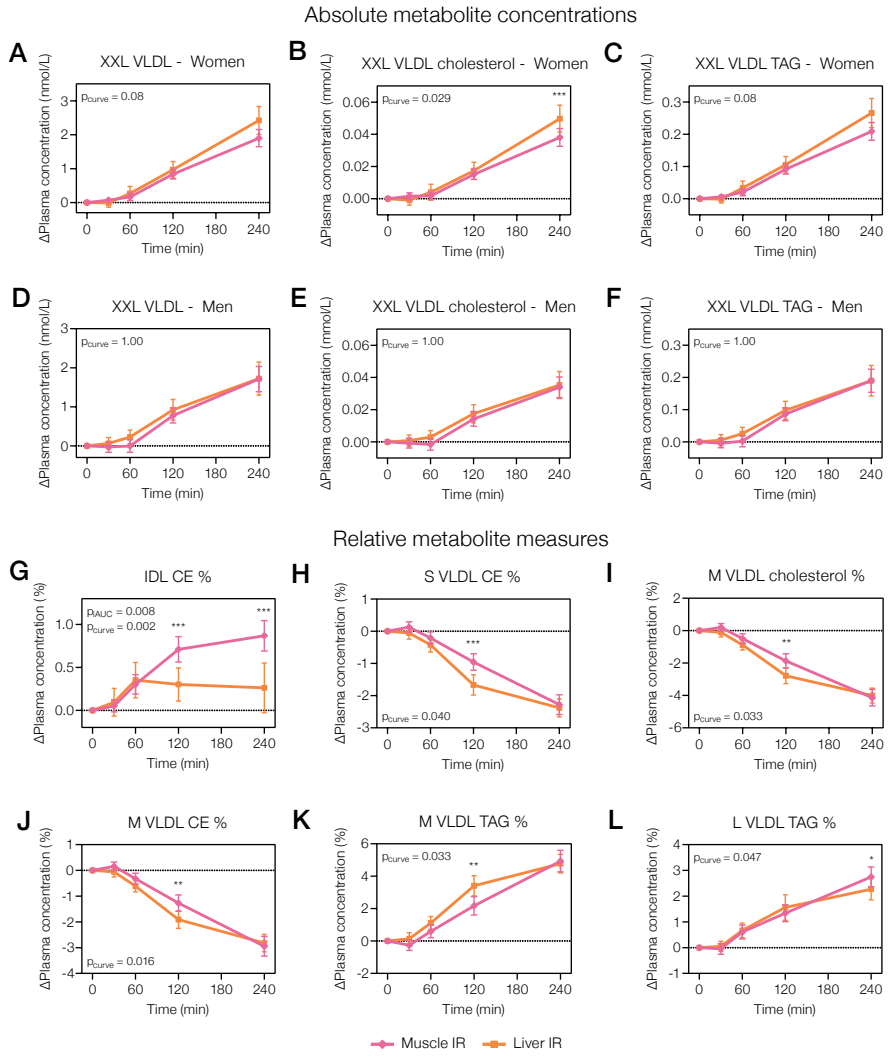

Figure S4. Plasma metabolite responses to a high-fat mixed meal. Responses were defined as change from fasting (value at postprandial timepoint – fasting value) and data are shown as means with 95% confidence intervals. Differences between liver IR and muscle IR were tested using linear mixed-effects models with adjustment for age, sex, center, BMI, and waist-to-hip ratio. Significant LSD post-hoc pairwise comparisons per timepoint are denoted with *($p < 0.05$), ** ($p < 0.01$) or ***($p < 0.001$).

6

Cardiometabolic health improvements upon dietary intervention are driven by tissue-specific insulin resistance phenotype: A precision nutrition trial

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Cell Metabolism **2023**, 35(1):71-83.e5

Highlights

- 242 adults with tissue-specific IR participated in a 12-week precision nutrition trial
- Health improvements upon the dietary intervention were driven by IR phenotype
- These cardiometabolic health improvements were independent of weight loss
- Precision nutrition based on IR phenotype enhances diet-induced health improvements

Summary

Precision nutrition based on metabolic phenotype may increase the effectiveness of interventions. In this proof-of-concept study, we investigated the effect of modulating dietary macronutrient composition according to muscle insulin-resistant (MIR) or liver insulin-resistant (LIR) phenotypes on cardiometabolic health. Women and men with MIR or LIR (n = 242, body mass index [BMI] 25–40 kg/m^2, 40–75 years) were randomized to phenotype diet (PhenoDiet) group A or B and followed a 12-week high-monounsaturated fatty acid (HMUFA) diet or low-fat, high-protein, and high-fiber diet (LFHP) (PhenoDiet group A, MIR/HMUFA and LIR/LFHP; PhenoDiet group B, MIR/LFHP and LIR/HMUFA). PhenoDiet group B showed no significant improvements in the primary outcome disposition index, but greater improvements in insulin sensitivity, glucose homeostasis, serum triacylglycerol, and C-reactive protein compared with PhenoDiet group A were observed. We demonstrate that modulating macronutrient composition within the dietary guidelines based on tissue-specific insulin resistance (IR) phenotype enhances cardiometabolic health improvements.
Clinicaltrials.gov registration: NCT03708419, CCMO registration NL63768.068.17.
Keywords: precision nutrition, dietary intervention trial, tissue-specific insulin resistance, metabotyping, glucose homeostasis, cardiometabolic health

Introduction

The unprecedented prevalence of obesity and related cardiometabolic disturbances calls for effective prevention strategies. A well-known strategy to improve cardiometabolic health is healthy nutrition, even in the absence of weight loss.[1,2] Nevertheless, a considerable proportion of individuals does not show clinically relevant improvements upon a dietary intervention.[3-5] These differential responses to diet may be explained by inter-individual heterogeneity in both exogenous and endogenous factors such as sex, dietary habits, gut microbiota composition, and metabolic phenotype.[6,7] Precision nutrition based on individual traits may increase the effectiveness of dietary interventions to improve metabolic health.[8]

There are indications that parameters related to glucose metabolism and insulin action or resistance, such as plasma glucose and insulin concentrations and indices based on these concentrations, may predict the response to dietary modification.[5,9,10] Importantly, insulin resistance (IR) can develop separately in insulin-sensitive tissues such as skeletal muscle and the liver, representing different etiologies toward cardio-metabolic diseases. We have recently shown that individuals with more pronounced liver IR (LIR) have a distinct metabolome,[11] lipidome,[12] adipose tissue transcriptome,[13] and systemic inflammatory profile[13] compared with individuals with more pronounced muscle IR (MIR). Therefore, individuals with these distinct tissue-specific IR phenotypes may respond differentially to dietary intervention.

Indeed, in a post hoc analysis of the CORDIOPREV-DIAB study, individuals with predominant MIR responded more favorably to a diet high in monounsaturated fatty acids (MUFAs), while individuals with predominant LIR responded more favorably to a low-fat, high-complex carbohydrate diet with regard to the disposition index, a composite marker of whole-body insulin sensitivity and insulin secretion.[14] In addition, both high-protein[15-17] and high-fiber diets,[18] as well as the Mediterranean diet,[19,20] have been shown to reduce liver fat content, which in turn may improve hepatic insulin sensitivity.[21,22] Furthermore, dietary fat quality may specifically impact skeletal muscle lipid metabolism and peripheral insulin sensitivity.[23] Importantly, however, well-designed, prospective, randomized, isocaloric dietary intervention trials to test the effectiveness of precision nutrition based on tissue-specific IR phenotype are currently lacking.

In this personalized glucose optimization through nutritional intervention (PERSON) study,[24] we investigated the efficacy of modulation of dietary macronutrient composition according to MIR and LIR phenotypes on parameters of glucose homeostasis, cardiometabolic health, health-related quality of life, and perceived well-being. We hypothesized that individuals with the MIR phenotype would benefit most from a diet rich in MUFA, and individuals with the LIR phenotype from a diet low in fat and rich in protein and fiber. Interestingly, these findings demonstrate that

individuals with the MIR phenotype showed a more pronounced cardiometabolic health improvement upon a low-fat, high-protein, and high-fiber (LFHP) diet, while individuals with the LIR phenotype had the greatest cardiometabolic health benefit from a high-MUFA (HMUFA) diet. Although not in concert with the initial hypothesis, these findings for the first time provide the proof-of-concept that modulating dietary macronutrient composition based on tissue-specific IR phenotype with healthy, isocaloric diets can induce more pronounced, clinically relevant improvements in cardiometabolic health, independent of changes in body weight.

Results

Study design and participant characteristics

Between May 2018 and November 2021, 990 men and women aged 40–75 years and with a body mass index (BMI) 25–40 kg/m^2 were enrolled, of whom 877 were fully screened for eligibility (Figure S1, CONSORT diagram). At screening, tissue-specific IR was assessed using the muscle insulin sensitivity index (MISI) and hepatic IR index (HIRI), which were calculated from the plasma glucose and insulin responses during a 7-point oral glucose tolerance test (OGTT).[25,26] Tertile cutoffs for MISI and HIRI from a previous study (the Maastricht study[11,27]) were used to identify individuals with predominant MIR or LIR.

In total, 242 participants (123 at Maastricht University Medical Center+ [MUMC+] and 119 at Wageningen University [WUR]) were included and randomized to phenotype diet (PhenoDiet) group A or B (n = 121 in both groups). PhenoDiet group A included individuals with MIR following an HMUFA diet and individuals with LIR following an LFHP diet. PhenoDiet group B included individuals with MIR following an LFHP diet and individuals with LIR following an HMUFA diet. The targeted macronutrient composition of both diets is described in Table S1. The dietary intervention strategy was based on weekly dietary counseling and provision of key products. Both diets were in line with the Dutch dietary guidelines, and we aimed for both diets to be eucaloric to keep participants on a stable body weight throughout the study. At baseline (week 0) and after 12 weeks of dietary intervention, participants underwent extensive metabolic phenotyping in a characterization week (Figure 1).

Overall, 58% of the randomized participants were women, mean age was 60 years, and mean BMI 29.9 kg/m^2. Baseline characteristics were well balanced in the two groups (Table 1). The majority of the participants (76%) was considered normal glucose tolerant at baseline according to fasting and 2-h glucose levels in response to an OGTT. Baseline characteristics with stratification for IR phenotype and diet intervention are described in Table S2. BMI was slightly higher in individuals with the LIR compared with MIR phenotype (p MIR versus LIR = 0.037) and the use of anti-inflammatory medication was higher in MIR compared with LIR (p MIR versus LIR = 0.041).

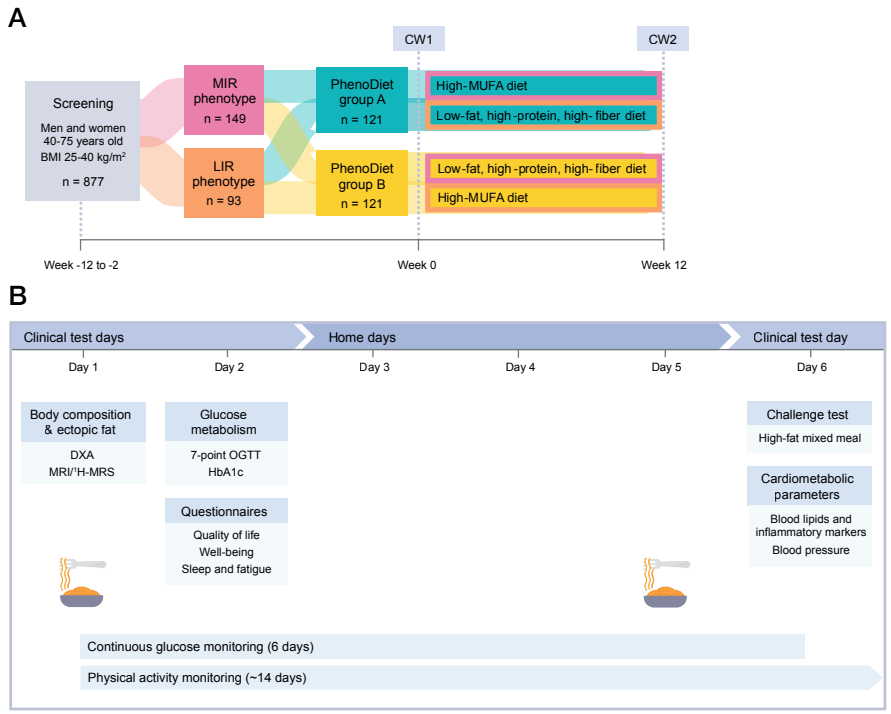

Figure 1. Study design of the PERSON study. (A) Tissue-specific insulin resistance was assessed at screening using a 7-point oral glucose tolerance test. Individuals with predominant muscle insulin resistance (MIR) or liver insulin resistance (LIR) were randomized to phenotype diet (PhenoDiet) group A or B. PhenoDiet group A consisted of individuals with MIR following a high-monounsaturated fatty acid (HMUFA) diet and individuals with LIR following a low-fat, high-protein, and high-fiber (LFHP) diet. PhenoDiet group B consisted of individuals with LIR following an HMUFA diet and MIR following an LFHP diet. (B) In clinical investigation week (CIW) 1 and 2, in weeks 0 and 12, respectively, participants underwent several clinical and at-home measurements.

In PhenoDiet group A, 94% (n = 114 of 121) and in PhenoDiet group B 88% (n = 107 of 121) completed the study (Figure S1). Twenty-two participants (13 in PhenoDiet group A, 9 in PhenoDiet group B) completed the study according to an adjusted protocol employed during the COVID-19 lockdown (only limited post-intervention measurements; STAR Methods). No major difference between the characteristics of completers and dropouts was observed at baseline (Table S3).

Table 1. Baseline characteristics of participants allocated to PhenoDiet group A or B

	PhenoDiet group A (n = 121)	PhenoDiet group B (n = 121)
MIR/LIR phenotype, n	76/45	73/48
Age, years	60 ± 8	60 ± 8
Women, n (%)	66 (54.5%)	75 (62.0%)
BMI, kg/m^2	30.1 ± 3.5	29.8 ± 3.5
Medication use, n (%)		
Antidepressants	5 (4.1%)	12 (9.9%)
Antihypertensives	27 (22.3%)	16 (13.2%)
Anti-inflammatory medication	14 (11.6%)	9 (7.4%)
Statins	9 (7.4%)	7 (5.8%)
Other	42 (34.7%)	37 (30.6%)
Family history of diabetes n (%)	22 (18.2%)	32 (26.4%)
Glucose status (%) (n = 240)		
NGT	94 (79.0%)	88 (72.7%)
IFG	5 (4.2%)	4 (3.3%)
IGT	12 (10.1%)	16 (13.2%)
Combined IFG/IGT	3 (2.5%)	4 (3.3%)
T2DM	5 (4.1%)	9 (7.4%)
Habitual physical activity, Baecke score	8.4 ± 1.2	8.3 ± 1.2
Employment status (%) (n = 236)		
Paid job	69 (59.5%)	55 (45.8%)
Retired	34 (29.3%)	43 (35.8%)
Other	13 (11.2%)	22 (18.3%)
Education level (%) (n = 235)		
Low	17 (14.7%)	18 (15.1%)
Intermediate	44 (37.9%)	48 (40.3%)
High	55 (47.4%)	53 (44.5%)

Values are n (%) or mean ± SD. MIR, muscle insulin resistance; LIR, liver insulin resistance; BMI, body mass index; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.

Habitual dietary intake at baseline was comparable between PhenoDiet group A and B

Self-reported habitual dietary intake before start of the intervention was assessed with a food frequency questionnaire (FFQ). After exclusion of data due to energy under- (n = 27) and overreporting (n = 1), FFQ data from 213 participants were included in these analyses. Habitual dietary intake was comparable between the groups, except for energy intake, which was higher in PhenoDiet group A (median [IQR]; 9.6 [7.8, 10.9] MJ) compared with PhenoDiet group B (8.6 [7.4, 10.6] MJ) (Table S4). Average intakes of calories from fat, protein, and carbohydrates were 37.7%, 15.6%, and 41.5%, respectively.

Adherence to the MHUFA and LFHP diets was high, with no differences between PhenoDiet group A and B

Compliance to the dietary interventions was evaluated with three 1-day food records that were randomly requested for throughout weeks 2–11 of the intervention via a mobile app,28 as well as with pre- and post-measurement of plasma fatty acid profile. After exclusion of data from 20 participants (MIR – HMUFA, n = 10; LIR – LFHP, n = 2; LIR – HMUFA, n = 4; MIR – LHFP, n = 4) due to energy underreporting, food record data from 206 participants were included in these analyses. Advised macronutrient composition of the two intervention diets and reported intake can be found in Table S5. Macronutrient composition of the two different intervention diets was comparable in PhenoDiet group A and B. Individuals randomized to the HMUFA diet reported higher intake of calories from total fat and MUFA, lower intake of protein and fiber, and similar intake of saturated fatty acid (SFA) and carbohydrates compared with those on the LFHP diet. The contribution of MUFA to total plasma fatty acid concentrations increased in individuals on the HMUFA diet, while it decreased in those on the LFHP diet (Table S6). Plasma SFA concentrations were reduced after both diets.

The change in the primary outcome disposition index was not significantly different between intervention groups

Glucose homeostasis and insulin sensitivity were assessed with a 7-point venous OGTT (75 g of glucose) before and at the end of the intervention. The primary outcome was the disposition index, which is a composite measure of insulin sensitivity and insulin secretion. The disposition index was 412 (369–460) (estimated marginal mean with adjustment for age, sex, and center [95% CI]) before intervention and 406 (365–451) after intervention in PhenoDiet group A, and 357 (321–398) before intervention and 380 (343–423) after intervention in PhenoDiet group B. Differences between groups did not reach statistical significance (p = 0.109, group × time) (Table 2; Figure 2A). Also, there was no change over time in either of the intervention groups (p = 0.640, time).

Table 2. Primary and secondary outcomes at baseline and after 12 weeks in PhenoDiet groups A and B

		PhenoDiet group A (n = 121)		PhenoDiet group B (n = 121)		*P*-value		
	n*	week 0	week 12	week 0	week 12	Group	Time	Group x Time
Primary outcome								
Disposition index (AU)	199	412 (369 - 460)	406 (365 - 451)	357 (321 - 398)	380 (343 - 423)	0.068	0.640	0.109
Secondary outcomes								
Glucose metabolism								
Fasting glucose (mmol/L)	199	5.3 (5.2 - 5.5)	5.3 (5.2 - 5.4)	5.5 (5.3 - 5.6)	5.3 (5.2 - 5.4)	0.179	0.146	0.238
Fasting insulin (pmol/L)	199	47.5 (44.0 - 51.4)	46.0 (42.4 - 49.9)	52.7 (48.9 - 56.9)	46.0 (42.4 - 49.9)	0.063	0.285	**0.019**
2-hour glucose (mmol/L)	199	6.1 (5.8 - 6.5)	6.2 (5.8 - 6.5)	6.5 (6.1 - 6.9)	6.1 (5.8 - 6.5)	0.123	0.561	**0.020**
2-hour insulin (pmol/L)	199	349.7 (308.3 - 396.3)	337.0 (297.9 - 381.1)	397.0 (350.8 - 449.8)	322.9 (285.1 - 365.6)	0.154	0.569	**0.023**
HOMA-IR (AU)	199	1.6 (1.5 - 1.8)	1.6 (1.4 - 1.7)	1.8 (1.7 - 2)	1.6 (1.4 - 1.7)	0.052	0.203	**0.017**
HOMA-β (AU)	199	76.5 (71.3 - 81.8)	76.2 (70.8 - 82)	79.8 (74.5 - 85.5)	73.9 (68.5 - 79.6)	0.301	0.931	0.079
Matsuda index (AU)	199	4.8 (4.4 – 5.3)	5.1 (4.6 – 5.6)	4.2 (3.9 – 4.6)	5.1 (4.6 – 5.5)	**0.032**	0.150	**0.004**
Insulinogenic index (AU)	199	32.2 (29.6 - 35)	30.4 (27.8 - 33.2)	32.3 (29.8 - 35.1)	28.8 (26.4 - 31.5)	0.957	0.072	0.234
MISI (AU)	191	0.123 (0.11 - 0.138)	0.130 (0.114 - 0.147)	0.116 (0.104 - 0.13)	0.151 (0.133 - 0.171)	0.424	0.583	**0.038**
HIRI (AU)	198	383 (348 - 421)	346 (311 - 385)	404 (367 - 444)	340 (305 - 378)	0.505	**0.021**	0.253
HbA1c (mmol/mol)	199	36.0 (35.2 - 36.7)	36.0 (35.4 - 36.7)	36.5 (35.7 - 37.2)	35.9 (35.2 - 36.6)	0.635	0.976	0.091
Anthropometrics								
Weight (kg)	221	86.9 (84.9 - 88.9)	85.2 (83.4 - 87.1)	87.9 (85.9 - 89.7)	85.5 (83.6 - 87.5)	0.408	**<0.001**	0.224
Waist circumference (cm)	221	101.2 (99.5 - 102.8)	99.1 (97.7 - 100.7)	102.4 (100.9 - 104)	100.5 (99.1 - 102.1)	0.187	**<0.001**	0.789
Waist-to-hip ratio	221	0.93 (0.92 - 0.94)	0.92 (0.91 - 0.94)	0.94 (0.93 - 0.95)	0.94 (0.92 - 0.95)	0.156	0.149	0.947
Body composition								
Body fat mass (%)	195	36.1 (35.2 - 37.1)	35.0 (34.0 - 36.1)	37.0 (36.1 - 37.9)	35.4 (34.4 - 36.4)	0.186	**<0.001**	0.078
Body fat mass (kg)	195	31.4 (30.1 - 32.8)	29.8 (28.4 - 31.3)	32.7 (31.3 - 34)	30.5 (29.1 - 31.9)	0.193	**<0.001**	0.058
Lean body mass (kg)	195	51.6 (50.6 - 52.7)	51.4 (50.4 - 52.5)	52.0 (51.1 - 53.1)	52.0 (50.9 - 53.1)	0.604	0.130	0.466
Android fat mass (kg)	195	3.2 (3 - 3.3)	3.0 (2.8 - 3.1)	3.3 (3.1 - 3.4)	3.0 (2.9 - 3.2)	0.399	**<0.001**	0.535
Gynoid fat mass (kg)	195	4.9 (4.6 - 5.1)	4.7 (4.4 - 4.9)	5.1 (4.9 - 5.4)	4.8 (4.6 – 5.0)	0.114	**<0.001**	**0.035**
Android/gynoid ratio	195	1.21 (1.18 - 1.23)	1.19 (1.16 - 1.22)	1.19 (1.16 - 1.21)	1.18 (1.15 - 1.21)	0.261	**0.031**	0.498
VAT (L)[a]	70	5.4 (4.9 – 6.0)	5.0 (4.5 - 5.5)	5.3 (4.8 - 5.8)	5.0 (4.6 - 5.5)	0.808	**<0.001**	0.489
VAT (cm^2)[b]	88	158 (146 - 170)	145 (134 - 158)	176 (163 - 191)	162 (149 - 176)	**0.047**	**<0.001**	0.972
Cardiometabolic parameters								
Total cholesterol (mmol/L)	198	5.3 (5.1 - 5.5)	4.8 (4.7 - 5)	5.4 (5.2 - 5.6)	4.8 (4.6 - 5)	0.432	**<0.001**	0.078
HDL cholesterol (mmol/L)	198	1.3 (1.2 - 1.3)	1.2 (1.2 - 1.3)	1.3 (1.2 - 1.3)	1.2 (1.1 - 1.2)	0.266	**<0.001**	0.101
Total cholesterol:HDL ratio	198	4.2 (4.0 - 4.4)	4.0 (3.8 - 4.2)	4.4 (4.2 - 4.6)	4.2 (4.0 - 4.4)	0.146	**<0.001**	0.980

Table 2. Continued

		PhenoDiet group A (n = 121)		PhenoDiet group B (n = 121)		*P*-value		
	n*	week 0	week 12	week 0	week 12	Group	Time	Group x Time
Cardiometabolic parameters								
TAG (mmol/L)	196	1.3 (1.2 - 1.4)	1.2 (1.2 - 1.3)	1.5 (1.4 - 1.6)	1.3 (1.2 - 1.4)	**0.033**	0.103	**0.028**
FFA (mmol/L)	196	0.5 (0.4 - 0.5)	0.4 (0.4 - 0.5)	0.5 (0.4 - 0.5)	0.4 (0.4 - 0.5)	0.884	**0.013**	0.684
SBP (mmHg)	198	123.7 (121.1 - 126.2)	121.5 (119.1 - 123.9)	126.5 (123.9 - 129.1)	121.6 (119.1 - 124.2)	0.137	**0.033**	0.077
DBP (mmHg)	198	77.9 (76.2 - 79.7)	76.3 (74.6 – 78.0)	79.4 (77.7 - 81.1)	77.1 (75.4 - 78.7)	0.257	**0.013**	0.495
Inflammatory profile								
CRP (mg/L)	197	0.98 (0.81 - 1.17)	0.97 (0.78 - 1.19)	1.12 (0.94 - 1.34)	0.88 (0.71 - 1.08)	0.298	0.892	**0.034**

* n represent number of individuals for whom data was available from both week 0 and week 12.
[a] At MUMC+, VAT was assessed using a whole-body MRI scan. [b] At WUR, VAT was assessed using single-slice MRI.
Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center. *P*-values <0.05 are highlighted in bold.
CRP, C-reactive protein; DBP, diastolic blood pressure; FFA, free fatty acid; HDL, high density lipoprotein; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; SBP, systolic blood pressure; TAG, triacylglyceride; VAT, visceral adipose tissue.

Greater improvements in insulin sensitivity and glucose homeostasis in PhenoDiet group B

Fasting insulin, 2-h glucose, 2-h insulin, and HOMA-IR decreased, and MISI increased significantly in PhenoDiet group B, but not in PhenoDiet group A (all $p < 0.05$, group × time) (Figures 2B-2E). The Matsuda index, which reflects whole-body insulin sensitivity, also increased significantly in PhenoDiet group B (from 4.2 [3.9-4.6] to 5.1 [4.6-5.5]) compared with PhenoDiet group A (from 4.8 [4.4-5.3] to 5.1 [4.6-5.6]) ($p = 0.004$, group × time) (Figure 2F). HIRI decreased significantly in both groups ($p = 0.021$, time), with no difference between the groups ($p = 0.25$, group × time). HbA1c tended to decrease slightly in PhenoDiet group B compared with PhenoDiet group A ($p = 0.091$, group × time) (Table 2). Additional statistical adjustment for weight change did not affect these results (data not shown).

We additionally compared changes in glucose and insulin areas under the curve (AUCs) in response to the OGTT between the two groups. The AUCs of postprandial glucose showed a larger reduction ($p = 0.004$, group × time) and a trend for larger reduction in postprandial insulin ($p = 0.076$, group × time) in PhenoDiet group B compared with PhenoDiet group A (Figure S2).

The greater improvements in insulin sensitivity and glucose homeostasis in PhenoDiet group B were observed both in individuals with the MIR and LIR phenotype

We performed post hoc analyses with stratification for IR phenotype for the outcomes with significant group × time interaction (Figure 3; Table S7). Fasting insulin, HOMA-IR, Matsuda index, and MISI improved in both individuals with the MIR and individuals with the LIR phenotype in PhenoDiet group B, whereas these parameters did not improve in individuals with either IR phenotype within PhenoDiet group A. Within PhenoDiet group B, 2-h glucose and insulin decreased significantly in the MIR group following the LFHP diet, but the decreases did not reach significance in the LIR group on the HMUFA diet.

Glycemic variability was not affected in either of the groups

In addition to measuring glucose parameters in response to a laboratory challenge test, we assessed glycemic variability in daily-life settings for 6 days using continuous glucose monitoring (CGM). Mean glucose, glucose standard deviation (SD), glucose coefficient of variation (CV) %, % glucose time in range 3.9–7.8 mmol/L, and mean amplitude of glucose excursions (MAGE) were not affected in either of the groups (Table 3).

Minor weight loss and reduction in body fat and ectopic fat in both groups

Body weight decreased to a similar extent in both groups, with ~2.0% and ~2.7% in PhenoDiet group A and B, respectively ($p < 0.001$, time; $p = 0.22$, group × time) (Table 2). We performed a dual X-ray absorptiometry (DXA) to assess body composition. The weight loss was caused by a reduction in body fat mass, which tended to be greater in PhenoDiet group B compared with PhenoDiet group A ($p = 0.058$, group × time). Both android and gynoid fat mass decreased in both groups ($p < 0.001$, time), but the reduction in gynoid fat mass was slightly larger in PhenoDiet group B, compared with PhenoDiet group A ($p = 0.035$, group × time). Additionally, at MUMC+, visceral adipose tissue (VAT), liver fat, and muscle fat were assessed using a whole-body magnetic resonance imaging (MRI) scan ,and at WUR, VAT was assessed using single-slice MRI and liver fat was measured using proton magnetic resonance spectroscopy (^{1}H-MRS). VAT decreased in both groups in both centers, without significant differences between groups ($p = 0.49$ [whole-body MRI] and 0.97 [single-slice MRI], group × time) (Table 2). Liver fat and muscle fat decreased to a similar extent in both groups, with no significant differences between groups ($p = 0.58$ [liver fat measured by MRI], 0.15 [liver fat measured by MRS], and 0.73 [muscle fat], respectively, group × time) (Table 3).

Larger reduction in serum TAG in PhenoDiet group B and similar reductions in cholesterol, FFA, and blood pressure in both groups

Both groups showed a decrease in fasting serum total cholesterol and high-density lipoprotein (HDL) cholesterol levels, with a tendency for a greater decrease in total cholesterol in PhenoDiet group B ($p < 0.001$, time; $p = 0.078$, group × time) (Table 2). Fasting serum triacylglycerol (TAG) decreased in PhenoDiet group B, whereas it did not change in PhenoDiet group A ($p = 0.028$, group × time) (Figure 2H). The lack of improvement in serum TAG in PhenoDiet group A was mainly driven by a lack of improvement of individuals with the MIR phenotype on the HMUFA diet (Figure 3G). Fasting free fatty acids (FFAs) decreased in both groups to a similar extent ($p = 0.013$, time; $p = 0.68$, group × time) (Table 2). Both interventions significantly reduced systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Table 2). The reduction in SBP tended to be larger in PhenoDiet group B ($p = 0.077$, group × time).

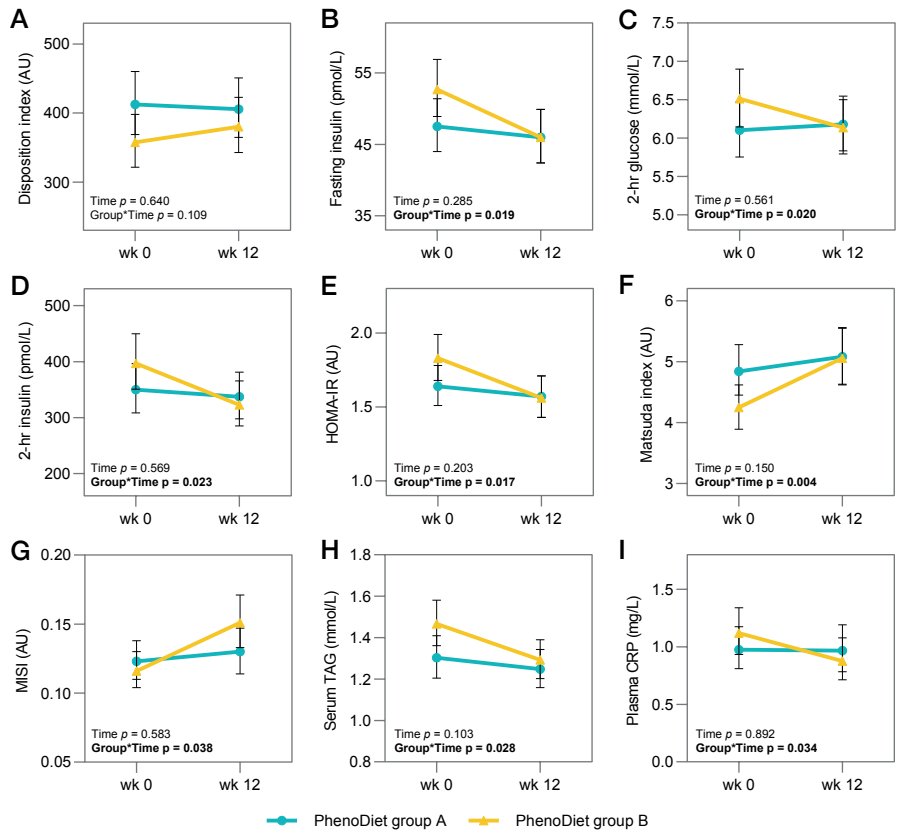

Figure 2. Greater improvements in insulin sensitivity, glucose tolerance, fasting TAG, and CRP in PhenoDiet group B compared with PhenoDiet group A. Individuals in PhenoDiet group B (n = 121) had more pronounced improvements in fasting insulin (B), 2-h glucose (C), 2-h insulin (D), HOMA-IR (E),Matsuda index (F), muscle sensitivity index (MISI) (G), serum triacylglycerol (TAG) (H), and plasma C-reactive protein (CRP) (I), but not disposition index (A), after 12 weeks of dietary intervention compared with PhenoDiet group A (n = 121). Data are presented as estimated marginal means with 95% confidence intervals, adjusted for age, sex, and center. $p < 0.05$ highlighted in bold. Intervention effects were tested using a repeated measures linear mixed model.

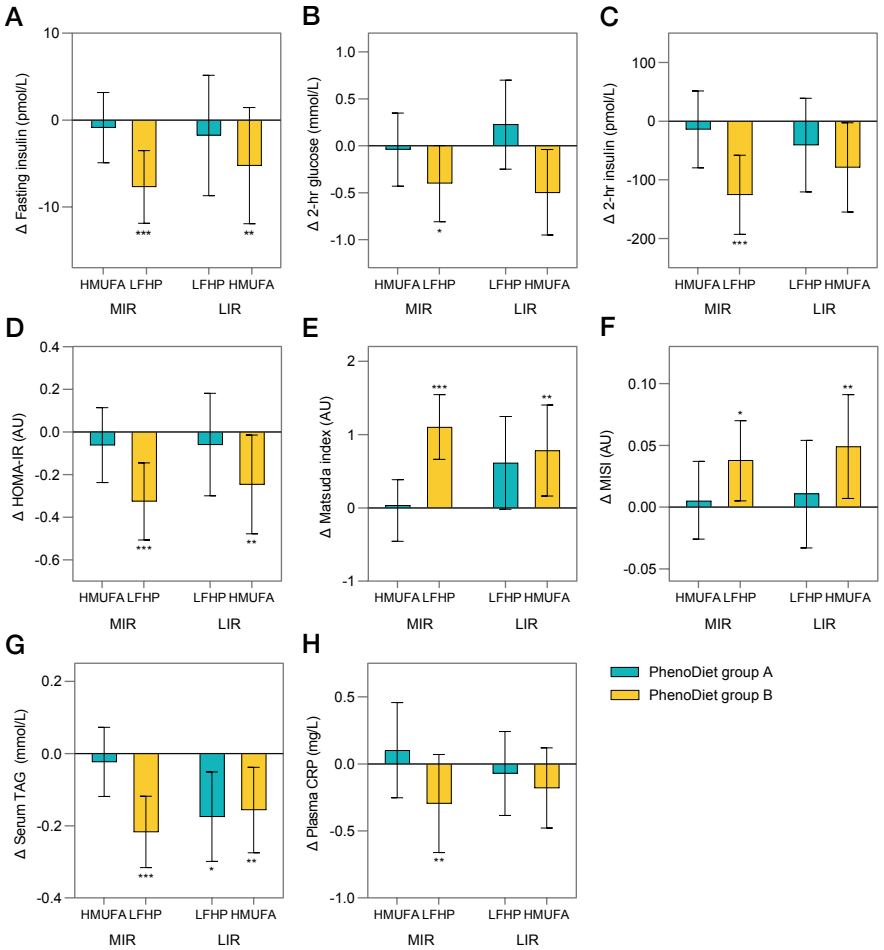

Figure 3. The greater improvements in PhenoDiet group B were observed both in the MIR and LIR phenotype. (A–F) Greater improvements in fasting insulin (A), 2-h glucose (B), 2-h insulin (C), HOMA-IR (D), Matsuda index (E), and MISI (F) were observed in PhenoDiet B (n = 121) in both individuals with MIR and LIR, whereas PhenoDiet A (n = 121) did not affect outcomes in either IR phenotype. (G) Serum TAG was reduced after 12 weeks in PhenoDiet group B in both individuals with MIR and LIR, and in PhenoDiet group A in LIR individuals only. (H) Plasma CRP was reduced in PhenoDiet group B in individuals with MIR and was not affected in the other groups. Data are presented as estimated marginal means with 95% confidence intervals, adjusted for age, sex, and center. *p < 0.05, **p < 0.01, ***p < 0.001 for time effect, as tested with a repeated measures linear mixed model, stratified for IR phenotype (post-hoc analysis).

Systemic inflammation marker CRP decreased only in PhenoDiet group B

Plasma C-reactive protein (CRP) decreased significantly from 1.12 (0.94–1.34) to 0.88 (0.71–1.08) mg/L in PhenoDiet group B, whereas it did not change in PhenoDiet group A (from 0.98 [0.81-11.7] to 0.97 [0.78-119]) (p = 0.034, group × time) (Table 2; Figure 2l). Post hoc analysis revealed that plasma CRP only improved in individuals with the MIR phenotype in the LFHP diet but did not significantly improve in other combinations of diet and phenotype (Figure 3H).

Similar reductions in postprandial glucose, insulin, TAG, and FFA upon a high-fat mixed meal in both groups

In addition to an OGTT, we also performed a liquid high-fat mixed meal (HFMM) test to assess postprandial responses to a meal containing fat, carbohydrates, and protein. The AUCs for postprandial glucose, insulin, and FFA response decreased for both interventions (all p < 0.05, time) without differences between PhenoDiet groups A and B (Figures S3 and S4). The postprandial increase in serum TAG decreased slightly in PhenoDiet group B compared with PhenoDiet group A, but this did not reach statistical significance (p = 0.11, group × time) (Figure S4).

The interventions had mixed effects on perceived well-being

Next to physiological measures, we included questionnaires to assess perceived well-being. Health-related quality of life was not affected in either of the groups (Table 3). Of the questionnaires related to sleep and fatigue, only the Epworth sleepiness scale score significantly decreased in both groups, indicating a reduction in daytime sleepiness, but with no difference between the groups (p = 0.044, time; p = 0.12, group × time). The Chalder fatigue score tended to decrease in PhenoDiet group B only, indicating a reduction in self-reported fatigue (p = 0.58, time; p = 0.090, group × time). The perceived stress score increased in PhenoDiet group B, indicating an increase in perceived stress compared with PhenoDiet group A (p = 0.003, group × time).

Light-intensity physical activity decreased slightly in both groups

Physical activity was objectively measured throughout ~7 days in free-living conditions at the start and end of the intervention period using a thigh-worn accelerometer. In both groups, light-intensity physical activity decreased from baseline to week 12, with no difference between the groups (p = 0.030, time; p = 0.23, group × time) (Table 3). Moderate-to-vigorous physical activity (MVPA) did not change in either of the groups.

Table 3. Secondary outcomes at baseline and after 12 weeks in PhenoDiet groups A and B

		PhenoDiet group A (n=121)		PhenoDiet group B (n=121)		*p*-value		
	n*	week 0	week 12	week 0	week 12	Group	Time	Group x Time
Glycemic variability								
Mean glucose (mmol/L)	211	6.0 (5.9 - 6.1)	6.0 (5.9 - 6.1)	6.2 (6.1 - 6.3)	6.1 (6 - 6.2)	**0.031**	0.545	0.178
SD glucose (mmol/L)	211	0.85 (0.80 - 0.91)	0.89 (0.83 - 0.94)	0.93 (0.88 - 0.99)	0.91 (0.86 - 0.97)	**0.046**	0.185	0.148
CV glucose (%)	211	14.2 (13.4 – 15.0)	14.8 (14.0 - 15.6)	15.1 (14.3 - 15.9)	15.0 (14.2 - 15.8)	0.115	0.134	0.227
Time in range 3.9-7.8 mmol/L (%)	211	93.6 (86.7 - 101.2)	93.3 (90.6 - 95.9)	84.3 (78 - 91)	89.6 (87.1 - 92.3)	0.055	0.887	0.102
MAGE (mmol/L)	211	2.1 (2 - 2.3)	2.2 (2.1 - 2.4)	2.4 (2.2 - 2.5)	2.3 (2.1 - 2.4)	**0.045**	0.438	0.159
Ectopic fat								
Liver fat (%) (MRI)[a]	69	5.2 (3.9 - 6.8)	3.4 (2.5 - 4.5)	6.1 (4.7 - 7.9)	4.2 (3.2 - 5.5)	0.367	**<0.001**	0.580
Liver fat (%) (^{1}H-MRS)[b]	84	2.6 (2.0 - 3.5)	1.3 (1.0 - 1.7)	3.2 (2.4 - 4.4)	1.3 (0.9 - 1.7)	0.347	**<0.001**	0.154
Muscle fat (%)	70	7.7 (7.2 - 8.2)	7.6 (7.1 - 8.1)	7.4 (7.0 - 7.9)	7.3 (6.9 - 7.8)	0.427	**0.036**	0.728
Physical activity								
LPA (h/day)	187	5.1 (4.8 – 5.3)	4.8 (4.6 – 5.1)	5.1 (4.9 – 5.4)	5.0 (4.7 – 5.3)	0.942	**0.030**	0.233
MVPA (h/day)	187	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.3)	0.562	0.241	0.297
Quality of life								
RAND-36 PCS	220	65.7 (64.2 - 67.3)	65.8 (64.3 - 67.4)	65.3 (63.8 - 66.9)	66.3 (64.6 - 67.9)	0.721	0.451	0.543
RAND-36 MCS	220	60.4 (58.9 - 61.9)	59.5 (58.2 - 60.9)	59.4 (58 - 60.9)	60.2 (58.8 - 61.6)	0.353	0.140	0.946
Sleep and fatigue								
Global PSQI score	220	5.0 (4.3 - 5.2)	5.1 (4.6 - 5.6)	4.7 (4.3 - 5.2)	5.2 (4.7 - 5.7)	0.700	0.189	0.534
Epworth Sleepiness Scale score	220	7.1 (6.4 - 7.7)	6.5 (5.8 - 7.1)	7.2 (6.6 - 7.9)	7.2 (6.4 - 7.9)	0.325	**0.044**	0.115
Chalder Fatigue score	220	11.7 (11.2 - 12.3)	11.4 (10.7 - 12.1)	11.7 (11.1 - 12.3)	11.1 (10.4 - 11.8)	0.892	0.576	0.090
Perceived stress								
Perceived Stress Score	220	8.8 (7.9 - 9.6)	8.2 (7.4 - 9)	8.6 (7.8 - 9.4)	9.4 (8.5 - 10.4)	0.333	0.592	**0.003**

* n represent number of individuals of which data was available from both week 0 and week 12.
[a] At MUMC+, liver fat was assessed using a whole-body MRI scan. [b] At WUR, liver fat was measured using ^{1}H-MRS.
Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center.
P-values <0.05 are highlighted in bold.
CV, coefficient of variation; LPA, light physical activity; MAGE, mean amplitude glucose excursion; MCS, mental component summary; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MVPA, moderate-to-vigorous physical activity; PCS, physical component summary; PSQI, Pittsburgh sleep quality index; SD, standard deviation.

Discussion

In this study, we show for the first time that improvements in cardiometabolic health after modulation of dietary macronutrient composition are dependent on tissue-specific IR phenotype. We defined two PhenoDiet groups, with PhenoDiet group A including individuals with MIR following HMUFA diet and individuals with LIR following a LFHP diet, and PhenoDiet group B including individuals with LIR following a HMUFA diet and MIR following a LFHP diet. The data demonstrate pronounced and clinically relevant improvements in insulin sensitivity, fasting plasma insulin and TAG concentrations, glucose tolerance, and CRP in PhenoDiet group B compared with PhenoDiet group A. These findings provide evidence for a greater effectiveness of a precision nutrition strategy based on tissue-specific IR phenotypes over a "one-size-fits-all" dietary approach within the general dietary guidelines in improving cardiometabolic health.

Here, we demonstrate for the first time in a prospective study that individuals with distinct tissue-specific IR phenotypes respond differentially to dietary macronutrient modification. Interestingly, peripheral, rather than hepatic, insulin sensitivity showed a distinct differential response between PhenoDiet groups A and B. The Matsuda index significantly improved by ~20% in PhenoDiet group B compared to ~5% in PhenoDiet group A. Besides MISI, 2-h glucose and 2-h insulin concentrations improved more in PhenoDiet group B, independent of IR phenotype, while no distinct responses between PhenoDiet groups A and B were observed for HIRI and fasting plasma glucose. The Matsuda index[29] and MISI[26] have previously been validated against the glucose disposal rate, as determined by the gold-standard two-step hyperinsulinemic-euglycemic clamp. Both indices represent primarily peripheral, or skeletal muscle, insulin sensitivity.

The underlying mechanisms for the more pronounced improvements in particularly peripheral insulin sensitivity and overall cardiometabolic health in individuals with the MIR phenotype on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet remain to be elucidated. Interestingly, modification of microbial composition by either fecal transplantation from lean donors to men with the metabolic syndrome, or dietary fiber intervention improved peripheral but not hepatic insulin sensitivity.[30,31] These data suggest that modulation of gut microbial composition may primarily affect peripheral insulin sensitivity, and this may thus be a putative underlying mechanism for the more pronounced effects on peripheral insulin sensitivity in individuals with the MIR phenotype on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet. The high content of slowly fermentable fibers in the LHFP diet (such as β-glucan, the fiber that was provided in the present LFHP diet) may ferment more distally in the colon, whereby the produced short-chain fatty acids (SCFAs) may bypass the liver and elicit metabolic effects more peripherally.[32] Additionally, high-fermented foods, including yogurt and quark (largely provided within the LFHP diet), can increase microbial diversity and decrease inflammatory markers.[33] Together, several components within the LFHP diet may have elicited improvements in peripheral insulin sensitivity and inflammation, possibly via modulation of the gut microbiota.

Despite these indications that microbial modulation may target peripheral insulin sensitivity specifically, a role of microbiota composition in hepatic metabolism, possibly depending on initial microbial composition as well as site of colonic fermentation, cannot be excluded.[34] A diet rich in MUFA and thereby rich in polyphenols may also affect microbial composition and liver lipid metabolism.[34-36] Besides that, we have previously shown that a meal high in PUFA or MUFA acutely decreased circulating VLDL-TAG levels (liver-derived TAG), increased the fractional synthetic rate of TAG in the skeletal muscle, and increased postprandial insulin sensitivity, compared with SFA.[23] In line, in this study the HMUFA diet reduced fasting TAG levels and tended to reduce postprandial TAG levels in individuals with LIR compared with MIR. These data suggest that the HMUFA diet may affect hepatic lipid metabolism, thereby possibly contributing to improved peripheral insulin sensitivity through inter-organ crosstalk.

The findings are in line with a recent post hoc analysis of the CORDIOPREV-DIAB study, which showed that individuals with distinct tissue-specific IR phenotypes benefit most from diets that differ in macronutrient composition.[14] Based on the CORDIOPREV-DIAB study, we hypothesized that individuals with the MIR phenotype would benefit more from an HMUFA diet and individuals with the LIR phenotype more from an LFHP diet. We, however, observed a nonsignificant tendency for an improved disposition index and a more pronounced improvement in cardiometabolic health in individuals with the MIR phenotype on an LFHP diet and individuals with the LIR phenotype on an HMUFA diet (PhenoDiet group B as compared with PhenoDiet group A). These conflicting findings may relate to several factors, including differences in study populations (overall more healthy population in the present PERSON study), in assessment of LIR, and in composition of diet interventions. These contrasting results illustrate the complexity of precision nutrition. Further advancement of the field of precision nutrition requires more well designed, clinical trials with deep phenotyping to better understand the mechanisms that underlie inter-individual variation in response to diet. Such studies are needed to identify the most important factors that explain individual response to diet, as well as to validate precision nutrition-based strategies.

Interestingly, 76% of both individuals with MIR and LIR were considered normal glucose tolerant at baseline. Nevertheless, based on elevated waist circumference, body fat percentage, and total cholesterol levels observed in this study population, individuals with MIR or LIR may already be at increased risk for metabolic perturbations

before the onset of disturbed glucose homeostasis as defined by established clinical cutoff values for impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Previous findings also show that tissue-specific IR phenotypes are related to disturbances in metabolome, lipidome, and inflammatory profiles.[11-13] An important finding in this study is that individuals in both study arms showed improvements in body composition, body fat distribution, ectopic fat, and several cardiometabolic parameters, regardless of intervention group (PhenoDiet group A or B) and without substantial weight loss (average weight loss: 2.3% or ~2 kg). To illustrate, liver fat decreased by more than 40% on average in the total population and total cholesterol levels decreased on average to values within the healthy range (<5.0 mmol/L). These results highlight the effectiveness and clinical relevance of a healthy diet in individuals with tissue-specific IR. Importantly, however, we demonstrate that health improvements can be remarkably enhanced when modulating dietary macronutrient composition based on tissue-specific IR phenotype.

We included questionnaires related to perceived well-being to explore the relationship between objective (clinical parameters) health and subjective health and well-being. Although slight changes in fatigue and perceived stress were observed, the effects on subjective health and well-being were not consistent. These findings suggest that improvements in cardiometabolic health were not reflected in detectable improvements in perceived well-being.

A major strength of this study is that it is the first to investigate the effects of modulating dietary macronutrient composition according to tissue-specific IR with a prospective, double-blind, randomized design in a large number of individuals. Another strength of this study is the classification of individuals by using only one measurement (7-point OGTT), paving the way for implementation of precision nutrition into clinical practice, although even more easily measurable biomarkers may be identified in future research. Finally, the dietary interventions were implemented by intensive dietary counseling and provision of key products. Dietary compliance was high, with substantial differences in reported MUFA, protein, and fiber intake between the HMUFA and LFHP diets, while keeping carbohydrate and SFA intake similar between the diets. The macronutrient composition that we aimed for was largely achieved in both diets, although reported MUFA and fiber intakes were slightly lower than advised in the HMUFA and LFHP diets, respectively. This may be due to either lower actual intake or misreporting.[37] Nevertheless, the two intervention diets clearly differed in key macronutrients, and both diets were a considerable modification to the participants' habitual diet.

In conclusion, we here demonstrate for the first time that clinically relevant improvements in cardiometabolic health after dietary macronutrient intervention are driven by IR phenotype, with the optimal macronutrient composition for each phenotype leading to a more pronounced improvement in cardiometabolic health, independent of weight loss. Our findings indicate that precision nutrition based on metabolic phenotype may be superior to a one-size-fits-all diet based on general guidelines with respect to improving cardiometabolic health.

Limitations of study

We acknowledge several limitations of this study. First, more individuals with the MIR phenotype were included in the study compared with LIR (149 versus 93). Due to equal distribution of phenotypes between PhenoDiet groups A and B, by design, more individuals followed the HMUFA diet in PhenoDiet group A and more LFHP in PhenoDiet group B. Still, post hoc analyses revealed that the more pronounced improvements in PhenoDiet group B as compared with PhenoDiet group A were driven by improvements in both individuals with the MIR on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet. Furthermore, it appeared that the individuals in PhenoDiet group A were by chance somewhat more insulin sensitive at baseline compared with PhenoDiet group B. Nevertheless, statistical adjustments for baseline differences were made, indicating that the conclusions of larger improvements observed in PhenoDiet group B cannot be explained by a more unfavorable metabolic profile at baseline. Tissue-specific IR was assessed with a 7-point OGTT. This method has been validated again the gold-standard hyperinsulinemic-euglycemic clamp technique.[25,26] Nevertheless, contrary to the highly standardized hyperinsulinemic-euglycemic clamp technique, OGTT-derived measurement of tissue-specific IR may partially be affected by biological processes associated with the oral ingestion of glucose, including differences in gastrointestinal factors, such as the rate of glucose absorption by the gut and the related incretin response.[38] Furthermore, glucose and insulin responses to an OGTT may be affected by an individual's body size, as the dose of ingested glucose is the same for all. In addition, all blood samples were taken from a venous forearm catheter. Therefore, it should be noted that the degree of forearm glucose uptake may have contributed to inter-individual variation in venous plasma glucose concentrations.[39] Importantly, however, we have shown that based on just one OGTT, regardless of whether we were truly able to distinguish LIR and MIR, we identified distinct metabolic phenotypes, which could be replicated in independent cohorts[11-13] and which in this prospective study responded differentially to dietary intervention. We hereby provide support for the efficacy of the clinical use of (7-point) OGTT-derived measures of metabolic heterogeneity. Finally, this study is a proof-of-concept study, focused on specific IR phenotypes that are prevalent in ~30% of the overweight population. Future research has to demonstrate whether more metabolic and IR phenotypes that respond differentially to dietary macronutrient modulation can be defined.

Acknowledgments

We would like to sincerely thank our study participants for participating in the PERSON study. We thank W. Sluijsmans, H. Aydeniz, Y. Essers, and N. Hoebers for the support with performing lab analyses and H. Fick, K. Manusama, M. Grootte Bromhaar, O. Paling, M. Ummels, A. Hoge, S. Bowser, L. Omary, and all students for their invaluable contribution to the execution of the study. We thank AMRA for their support with the MRI analysis at UM and Medtronic for support with the CGMs. The project is organized by and executed under the auspices of TiFN, a public-private partnership on precompetitive research in food and nutrition. Funding for this research was obtained from the industry partners DSM Nutritional Products, FrieslandCampina, Danone Nutricia Research, AMRA Medical AB, and the Top-sector Agri&Food. We thank the industry partners for their in-kind and in-cash contribution. Graphical abstract created with BioRender.com.

Author contributions

I.T., A.G., and K.M.J. drafted the manuscript and were responsible for data analysis and execution of the study. G.B.H. was responsible for data management of the study. B.E. supported in the data processing and analysis. L.W. contributed to data collection and contributed to data analysis related to physical activity. E.S. was responsible for setting up the dietary intervention protocol and dietetics support during the study. E.E.B. was project leader and principal investigator and obtained funding for the project. E.E.B., L.A.A., G.H.G., E.J.M.F., D.H.J.T., and I.C.W.A. co-designed the study. All authors actively participated in project development, discussion of results, and revision of the article, and approved the final version of the manuscript.

Declaration of interests

S.P. is an employee at DSM Nutritional Products, C.M.S.-P. is an employee at FrieslandCampina, and J.d.V.-v.d.B. is an employee at Danone Nutricia Research.

Inclusion and diversity

We support inclusive, diverse, and equitable conduct of research.

Star methods

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ellen Blaak (e.blaak@maastrichtuniversity.nl).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The published article and supplemental information include the data used to generate the figures in the paper (Data S1). This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Experimental model and subject details

The PERSON study (PERSonalized glucose Optimization through Nutritional intervention) was a two-center, randomized, double-blinded, 12-week dietary intervention study with a parallel design (Fig. 1). The rationale and methodology of the PERSON study have been described in detail previously.[24] The study was conducted from May 2018 until November 2021 at Maastricht University Medical Center+ (MUMC+) and Wageningen University (WUR) in the Netherlands, in line with the principles of the Declaration of Helsinki. The protocol was approved by the Medical Ethical Committee of the MUMC+(NL63768.068.17) and registered at ClinicalTrials.gov (NCT03708419). All participants gave written informed consent.

Study participants

Participants were recruited via a volunteer database, flyers, and advertisements in local and online media. Inclusion criteria were: age 40–75 years, BMI 25–40 kg/m^2, body weight stability for at least 3 months (no weight gain or loss >3 kg), and tissue-specific IR, characterized as predominant LIR or MIR, as assessed by a 7-point oral glucose tolerance test (OGTT) based on venous plasma glucose and insulin concentrations. Exclusion criteria included among others pre-diagnosis of type 2 diabetes mellitus (T2DM), diseases or use of medication that affect glucose and/or lipid metabolism, major gastrointestinal diseases, history of major abdominal surgery, uncontrolled hypertension, smoking, alcohol consumption >14 units/week, and >4 h/week moderate-to-vigorous physical activity.[24]

Assessment of eligibility

Compliance with in- and exclusion criteria was assessed according to standard protocols during a screening visit as described previously.[24] Data on demographics, medical history, family history of DM (≥1 first-degree relative with DM), and medication use were collected by a screening questionnaire. Education level was categorized into low (no education, primary education, lower or preparatory vocational education, lower general secondary education), medium (intermediate vocational education, higher general senior secondary education or pre-university secondary education) and high (higher vocational education, university).

Tissue-specific IR was assessed based on the plasma glucose and insulin concentrations during a 7-point OGTT. Participants ingested 200 ml of a ready-to-use

75 g glucose solution (Novolab) within 5 min, and blood samples were collected from the antecubital vein via an intravenous cannula under fasting conditions (t = 0 min) and after ingestion of the glucose drink (t = 15, 30, 45, 60, 90, and 120 min) for determination of plasma glucose and insulin concentrations. LIR and MIR were estimated using calculations for the hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI) respectively, by Abdul-Ghani and colleagues.[26] The MISI calculation has been optimized using the cubic spline method.[25] HIRI and MISI were calculated as follows:

HIRI = glucose 0-30 [AUC in mmol/L x h] x insulin 0-30 [AUC in pmol/L x h]

MISI = (dGlucose/dt) / insulin [mean during OGTT in pmol/L]

In the calculation for MISI, dGlucose/dt is the rate of decay of plasma glucose concentration (mmol/L) during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir. Deviating glucose curves that were flagged by the calculator were visually inspected for MIR and LIR classification. Individuals were classified as “No MIR/LIR,” “MIR,” “LIR,” or “combined MIR/LIR,” using tertile cutoffs for MISI and HIRI. The lowest tertile of MISI represented individuals with MIR, while the highest tertile of HIRI represented individuals with LIR. The cutoffs for these tertiles were based on values of a selected study population of The Maastricht Study[27], which resembles the target population of the PERSON study. After inclusion of 163 participants, the median HIRI of the current study screening population was used for classification due to an apparent discrepancy in LIR prevalence between the two populations. Additional OGTT-derived indices and other outcomes were determined as described below and as previously reported.[24] Eligible participants started the study within 3 months after screening.

Randomization

Eligible participants were randomly assigned to either PhenoDiet group A or PhenoDiet group B, which consisted of unique combinations of the MIR and LIR metabolic phenotypes and two distinct diets meeting the Dutch dietary guidelines[40]. PhenoDiet group A included individuals with MIR following a high-monounsaturated fatty acids (HMUFA), and individuals with LIR following a low-fat, high-protein, and high-fiber (LFHP) diet. PhenoDiet group B included individuals with LIR and MIR on HMUFA and LFHP diets, respectively.

Random allocation to either PhenoDiet group A or B in 1:1 ratio was conducted by an independent researcher using center-specific minimization[41,42], with randomization factors of 1.0 for the LIR/MIR phenotype, and 0.8 for age and sex, and a base probability of 0.7 by means of biased-coin[43]. Both researchers and participants were blinded to the participants’ metabolic phenotype (LIR or MIR), and thus blinded to whether participants were allocated to PhenoDiet A or B.

Method details

Dietary intervention

The HMUFA diet had a targeted macronutrient composition of 38% of energy from fat (20% MUFA, 8% PUFA, 8% SFA), 48% of energy from carbohydrates (CHO) (30% polysaccharides; 3 g/MJ fiber), and 14% of energy from protein. The macronutrient composition of the LFHP diet was targeted at 28% of energy from fat (10% MUFA, 8% PUFA, 8% SFA), 48% of energy from CHO (30% polysaccharides; >4 g/MJ fiber), and 24% of energy from protein (Table S1). Energy from CHO was similar between diets. Key products that largely distinguished the two diets with regards to macronutrient composition were provided in pre-measured amounts. For the HMUFA diet, key products included olive oil, olives, olive tapenade, and low-fat margarine with olive oil. Key products for the LFHP diet included low-fat yogurt and quark, reduced-fat cheese, very low-fat spread, pumpkin seeds, baking margarine with olive oil, and a dietary fiber supplement (2 g β-glucan per 6 g, DSM Nutritional Products, Basel, Switzerland) providing 6–12 g of additional fiber per day. Participants were instructed to consume a certain amount of every provided product each day. Apart from the fiber supplement, all products were commercially available. Alcohol consumption was restricted to ≤ 1 glass/day, in agreement with the current Dutch dietary guidelines.[40]

Participants were assigned to one of eight energy groups ranging from 6 to 13 MJ/d according to their estimated individual energy requirement, which was calculated by averaging self-reported energy intake from a food frequency questionnaire (FFQ)[44] with the product of the predicted BMR, as calculated with Schofield equations[45], and self-reported physical activity level.

Individual counselling sessions with a dietician or research nutritionist were scheduled weekly at the research facilities to monitor adherence to the diet, adverse events and body weight to assess weight stability. Additional support was provided via e-mail or phone if needed. In case of weight instability, the participant’s energy group was adjusted to avoid further weight change. During the period of COVID-19 restrictions, all counseling sessions took place via phone or video call. The dietary intervention strategy has been described in more detail before.[24]

Dietary compliance

During the 12-week intervention, dietary compliance was assessed by three unannounced 1-day food records on two non-consecutive weekdays and one weekend day using the mobile app “Traqq”[28]. In addition, plasma fatty acid profile was measured by nuclear magnetic resonance spectroscopy as a biomarker for MUFA, PUFA and SFA consumption.[46]

Habitual dietary intake

A validated 163-item semi-quantitative FFQ[44] was used to assess habitual dietary intake before the start of the dietary intervention period. Dietary misreporting was evaluated by Goldberg's method,[47,48] using the ratio of daily energy intake (EI) to estimated basal metabolic rate (BMR). Energy under- (EI/BMR < 0.87) and over reporters (EI/BMR > 2.75) were excluded from data analyses.

Measurements

In the week before start of the dietary intervention (baseline) and in the last week of the 12-week intervention (week 12), participants were extensively phenotyped during a characterization week. This week included three or four (depending on study center and participation in additional subgroup measurements) clinical test days including a broad spectrum of laboratory analyses and three at-home days for additional data collection in daily-life settings.[24] On the clinical test days, participants were instructed to travel to the facility by car or public transport. The day prior to and during the characterization weeks, participants were requested to refrain from alcohol and vigorous physical activity.

7-point oral glucose tolerance test

A 7-point OGTT was performed according to the same procedures as during the screening visit. Participants consumed a standardized low-fat macaroni meal (30% of energy intake [en%] fat, 49 en% CHO, 21 en% protein; 1,560–2,460 kJ, depending on energy group) the evening before the OGTT, after which they remained fasted until the OGTT.

The primary outcome disposition index was calculated as: [Matsuda index * (AUC30 min insulin/AUC30 min glucose)], where AUC30 min is the area under the curve between baseline and 30 min of the OGTT for insulin (pmol/L) and glucose (mmol/L) as calculated using the trapezoidal method, respectively. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as (fasting glucose [mmol/L] × fasting insulin [mU/L])/22.5.[49] HOMA of β-cell function (HOMA- β) was calculated as (20 × fasting insulin [mU/L])/(fasting glucose [mmol/L] – 3.5). Matsuda index was defined as: [10,000 ÷ square root of [fasting plasma glucose (mg/dL) × fasting insulin (mU/L)] × [mean glucose (mg/dL) x mean insulin (mU/L)]], using glucose and insulin values of time points 0, 30, 60, 90, and 120 min.[29] Criteria of the WHO[50] were used define glucose status: normal glucose tolerance (NGT), fasting glucose <6.1 mmol/L and 2-hour glucose <7.8 mmol/L; impaired fasting glucose (IFG), fasting glucose 6.1 – 6.9 mmol/L and 2-hour glucose <7.8 mmol/L; impaired glucose tolerance (IGT), fasting glucose <6.1 mmol/L and 2-hour glucose 7.8 – 11.0 mmol/L; combined IFG/IGT, fasting glucose 6.1 – 6.9 mmol/L and 2-hour glucose 7.8-11.0 mmol/L; T2DM, fasting glucose ≥7.0 mmol/L and/or 2-hour glucose ≥11.1 mmol/L.

High-fat mixed-meal challenge test

A high-fat mixed-meal challenge test was performed at least 4 days after the OGTT, to determine the effects of the diets on postprandial glucose and lipid metabolism after a high-fat challenge. Participants consumed the same standardized low-fat macaroni meal as before the OGTT, after which they fasted 12 hours overnight. The liquid HFMM (350 g containing 2.8 MJ, 49 g [64 en%] fat, 48 g [29 en%] CHO, 12 g [7 en%] protein) was prepared in the university kitchen using whipped cream ice cream, whipped cream, full-fat milk, and sugar. An intravenous cannula was inserted in the antecubital vein for blood sampling. At least 30 min following insertion of the catheter, a fasting blood sample was drawn (t = 0 min). Subsequently, participants were asked to consume the liquid HFMM within 5 min and postprandial blood samples were drawn at t = 30, 60, 90, 120, 180, and 240 min for determination of glucose, insulin, free fatty acids (FFA) and triacylglycerol (TAG). Total cholesterol and HDL cholesterol were determined in fasting serum.

Body composition, fat distribution and ectopic fat deposition

Measurements of body weight and waist and hip circumference were performed according to standardized measurements.[24] Whole-body and regional fat mass, fat percentage, and lean body mass were assessed using dual-energy X-ray absorptiometry (DXA), while participants were fasted for ≥2 h (MUMC+, Discovery A, Hologic; WUR, Lunar Prodigy, GE Healthcare).

Fat distribution and ectopic fat deposition were assessed using magnetic resonance imaging (MRI) and/or magnetic resonance spectroscopy (MRS). At MUMC+, a whole-body scan was made after a ≥2 h fast with a 3T MRI scanner (3T MAGNETOM Prisma fit, Siemens Healthcare), using a radiofrequency transmit/receive body coil at Scannexus, Maastricht, the Netherlands. Analyses were performed using a computational modeling method [AMRA Medical AB, Linköping, Sweden] for quantification of visceral adipose tissue (VAT), intrahepatic lipid content (IHL), and muscle fat infiltration (MFI) in the anterior thighs. At WUR, IHL and abdominal fat were assessed with proton magnetic resonance spectroscopy (^{1}H-MRS) and MRI, respectively, on a 3T whole-body scanner (Siemens, Munich, Germany; Philips Healthcare, Best, the Netherlands from November 2020 onwards). MRI measurements were performed after a ≥2 h fast at hospital Gelderse Vallei, Ede, the Netherlands. Spectra for determination of IHL were obtained from a 30 × 30 × 20 mm voxel placed in the right lobe of the liver, avoiding blood vessels and bile ducts. Participants were instructed to hold their breath when spectra were acquired to reduce respiratory motion artifacts. Spectra were post-processed and analyzed using the AMARES algorithm in jMRUI software[51]. VAT was quantified in single-slice axial T1-weighted spin echo transverse images at the inter-vertebral space L3-L4 using the image analysis software program c (version 5.0, Tomovision).

Continuous glucose monitoring

Participants wore a continuous glucose monitor (CGM) for 6 days during characterization weeks 1 and 2. The CGM device (iPro2 and Enlite Glucose Sensor; Medtronic, Tolochenaz, Switzerland) was worn lateral to the umbilicus and recorded subcutaneous interstitial glucose values every 5 minutes. Participants were asked to perform four daily capillary glucose self-measurements (SMBG) via Contour XT (Ascensia Diabetes Care, Mijdrecht, the Netherlands) while wearing the CGM device. The CGM data files were then calibrated retrospectively using the SMBG values in CareLink (Medtronic, Tolochenaz, Switzerland) according to manufacturer's instructions. To avoid insufficient calibration, sensor glucose readings outside the time interval of the first and last SMBG measurements were excluded from the analysis. Participants were blinded to the CGM recording, but not to the SMBG values. In addition, CGM data files with irregular measurement frequencies (i.e. other than 5 minute) were excluded from the analysis (n = 3). The iglu package[52] (version 3.3.0) in R (version 4.0.2) was used to calculate mean glucose, standard deviation (SD), coefficient of variation (CV), time in range (between 3.9 and 7.8 mmol/L; TIR) and mean amplitude of glycemic excursions (MAGE).

Blood pressure

Systolic and diastolic pressure were measured in triplicate on the non-dominant arm with an automated sphygmomanometer after a 5-minute rest. The first measurement was used to acclimatize the subject to the measurements, and therefore omitted from the data.

Physical activity monitoring

Physical activity was assessed with the activPAL3 micro triaxial accelerometer (PAL Technologies Ltd., Glasgow, UK). The monitor was worn continuously attached to the anterior thigh, in the middle between the knee and the greater trochanter for ~14 days during both the characterization weeks, of which ~7 days in free-living conditions. Parameters of physical activity were quantified with a modified version of a home-written script[53], using sleeping and waking times recorded by the participants as input. We distinguished light-intensity physical activity (LPA) and moderate-to-vigorous physical activity (MVPA). LPA includes standing and stepping times with Metabolic Equivalent of Task (MET) values <3.[53] MVPA includes activities with MET values ≥3. Both measures were determined in hours per day. In the present study, only LPA and MVPA during the free-living days were used because physical activity during the characterization weeks with university visits and measurements is not reflective of regular physical activity level.

Self-reported sleep, well-being, and physical (in-)activity

General perceived health was assessed by the Physical and Mental Component Summary (PCS and MCS) scores obtained from the RAND-36[54]. Perceived stress was assessed with the 10-item Perceived Stress Scale (PSS-10)[55]. Physical and mental fatigue were assessed using the 14-item Chalder fatigue scale[56]. Sleep quality was assessed with the 10-item Pittsburgh Sleep Quality Index[57]. Daytime sleepiness was assessed with the 8-item Epworth Sleepiness scale[58]. Self-reported habitual physical activity and sedentary behavior were assessed using the Baecke questionnaire[59].

Adjusted COVID-19 protocol

Due to strict Dutch COVID-19 restrictions from March to June 2020, post-intervention measurements of 22 individuals were performed according to an adjusted protocol. The protocol included CGM measurements, anthropometric measurements and questionnaires as described above. The participants performed the measurements at home under guidance of the researcher via video connection. All other measurements were not performed during this period. The dietary intervention part of the study was completed according to the original protocol. The COVID-19 protocol was approved by the Medical Ethical Committee of the MUMC+ and participants gave their written informed consent.

Biochemical analyses of blood samples and biobanking

Venous blood was collected in EDTA tubes (Becton Dickinson, Eysins, Switzerland), which were centrifuged at 1,200 g, 4°C for 10 min and plasma was aliquoted subsequently. Serum tubes were left at room temperature for at least 30 min to allow clotting after sampling and centrifuged at 1,200 g, 20°C for 10 min before aliquoting of serum. All biological samples were snap-frozen in liquid nitrogen and stored at −80°C until analysis. Samples from both centers were analyzed at central laboratories. Plasma glucose, insulin, and FFA were measured on a Cobas Pentra C400 using ABX Pentra Glucose HK CP reagens (Horiba ABX Diagnostics, Montpellier, France), ELISA (Meso Scale Discovery, Gaithersburg, USA), and NEFA HR reagens (Wako chemicals, Neuss, Germany), respectively. Serum TAG, total cholesterol, and HDL cholesterol were measured on a Cobas Pentra C400 using ABX Pentra Triglycerides HK CP reagens, ABX Pentra Cholesterol CP reagens, and ABX Pentra HDL Direct, respectively. A fasting blood sample was drawn for determination of glycated hemoglobin (HbA1c) by the hospital laboratories of MUMC+ and Ziekenhuis Gelderse Vallei, Ede, the Netherlands. The inflammatory marker C-reactive protein (CRP) was measured in fasting plasma using a Luminex immunoassay performed by DSM Nutritional Products (Kaiseraugst, Switzerland).

Quantification and statistical analysis

Power and sample size

A total sample size of 202 was previously calculated to be required to detect a standardized effect size of 0.46 with a power of 90%.[24] Due to practical issues related to the unforeseen COVID-19 pandemic, 199 individuals completed the measures related to the primary outcome the disposition index. Participants for whom data that was missing due to the adjusted COVID-19 protocol were not considered dropouts but were excluded from the analyses related to these missing data to limit interference with study outcomes.

Statistical analyses

The number of dropouts between the two intervention groups was not significantly different ($p = 0.11$), and baseline characteristics did not differ between dropouts and completers (all $p > 0.05$) (Table S3). An intention to treat (ITT) analysis, which assumes that data was missing at random, was performed using a mixed-model with repeated measures to test intervention effects on primary and secondary parameters comparing PhenoDiet groups A and B. The model included age, sex, and study center as covariates, and time (baseline and week 12) as repeated measure. *Post-hoc* analyses with stratification for IR phenotype were performed in case of a significant group *x* time interaction. Estimated marginal means with 95% confidence intervals adjusted for the covariates are reported. For OGTT and high-fat mixed-meal responses, the AUC was calculated using the trapezoid method. Baseline characteristics were compared between the MIR and LIR phenotype, and between the diet groups within MIR and LIR groups using independent samples T-test for numerical data (mean ± SD) and using Fisher's exact test for categorical data (%).

Model assumptions were tested by plotting residual and predicted values and by visually inspecting residual Q-Q plots, to test homogeneity of variances and normality of residuals, respectively. Skewed variables were log-transformed (log10) to improve normality. Two-tailed $p < 0.05$ was considered statistically significant. Analyses were performed using IBM® SPSS® Statistics software version 28.

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Supplemental information

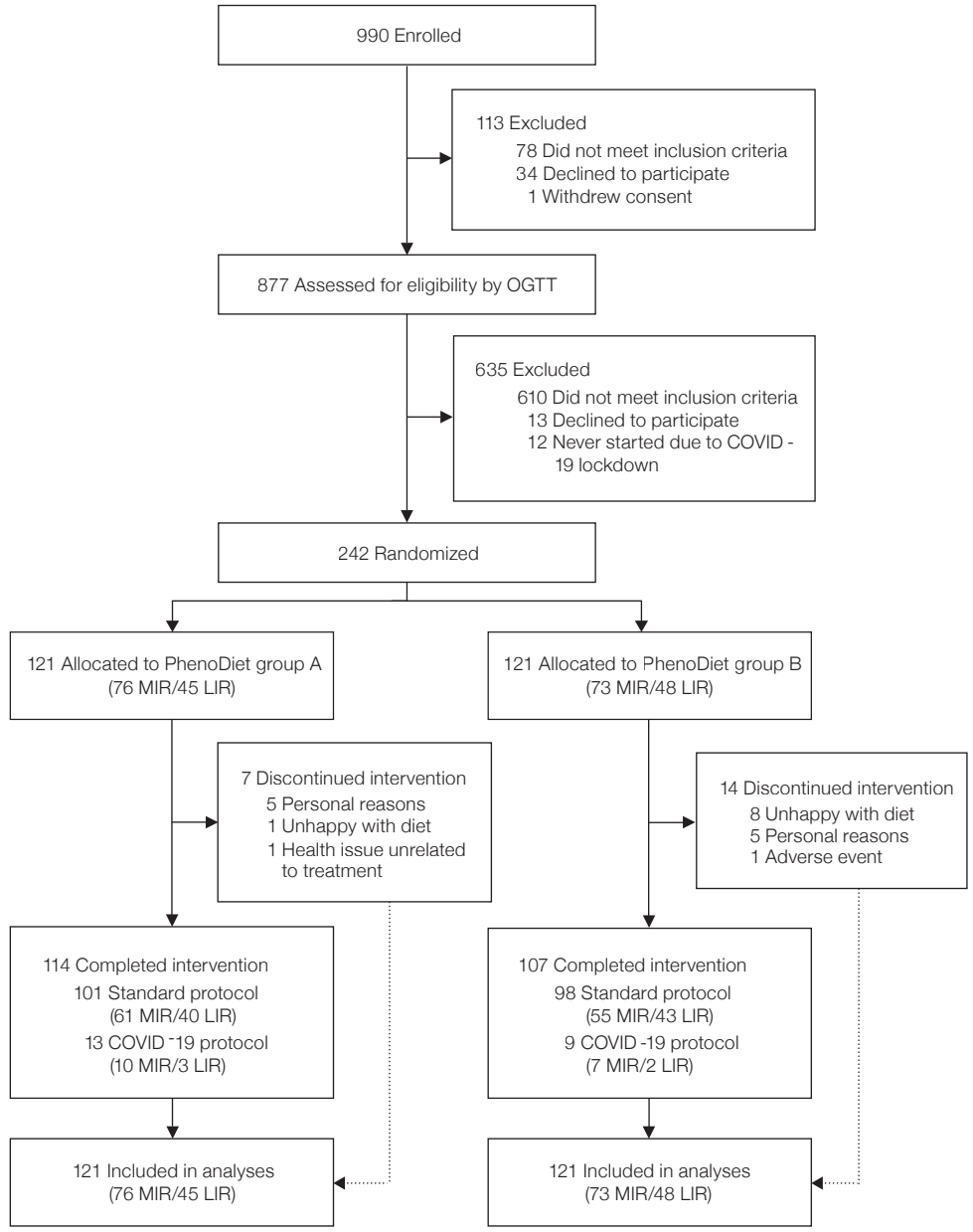

Figure S1. CONSORT diagram of participant enrolment and eligibiltiy, Related to STAR Methods.

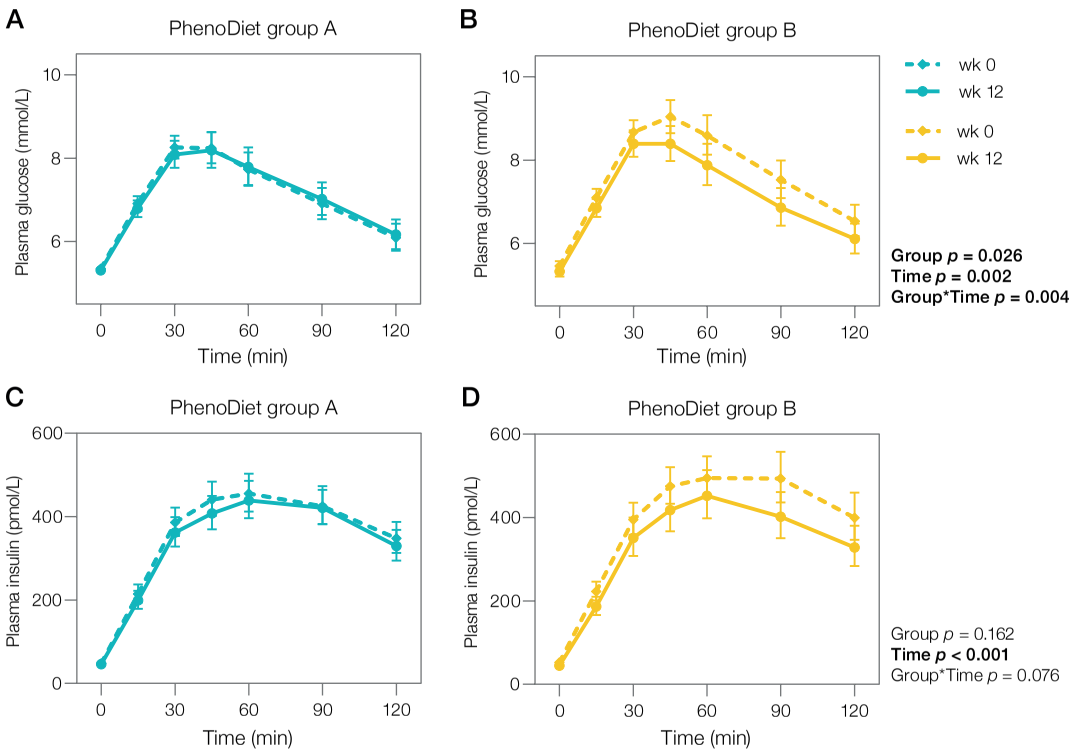

Fig S2. Plasma glucose and insulin concentrations in response to a 75g oral glucose tolerance test, Related to Table 2 and Figure 2. Plasma glucose concentrations in PhenoDiet group A (A) and B (B), and plasma insulin concentrations in PhenoDiet group A (C) and B (D) in response to an oral glucose tolerance test at week 0 and week 12. Dashed lines indicate week 0, solid lines week 12. Data are presented as geometric means with 95% CI and differences in area under the curves (AUCs) between PhenoDiet group A and B were assessed using a linear mixed model with repeated measures with adjustments for age, sex, and center. The AUCs of both postprandial glucose and insulin decreased in both groups (*P* for time all < 0.05) with a larger reduction in postprandial glucose (*P* for group x time = 0.004) and a trend for larger reduction in postprandial insulin (*P* for group x time = 0.076) in PhenoDiet group B compared to PhenoDiet group A. *P*-values <0.05 are highlighted in bold.

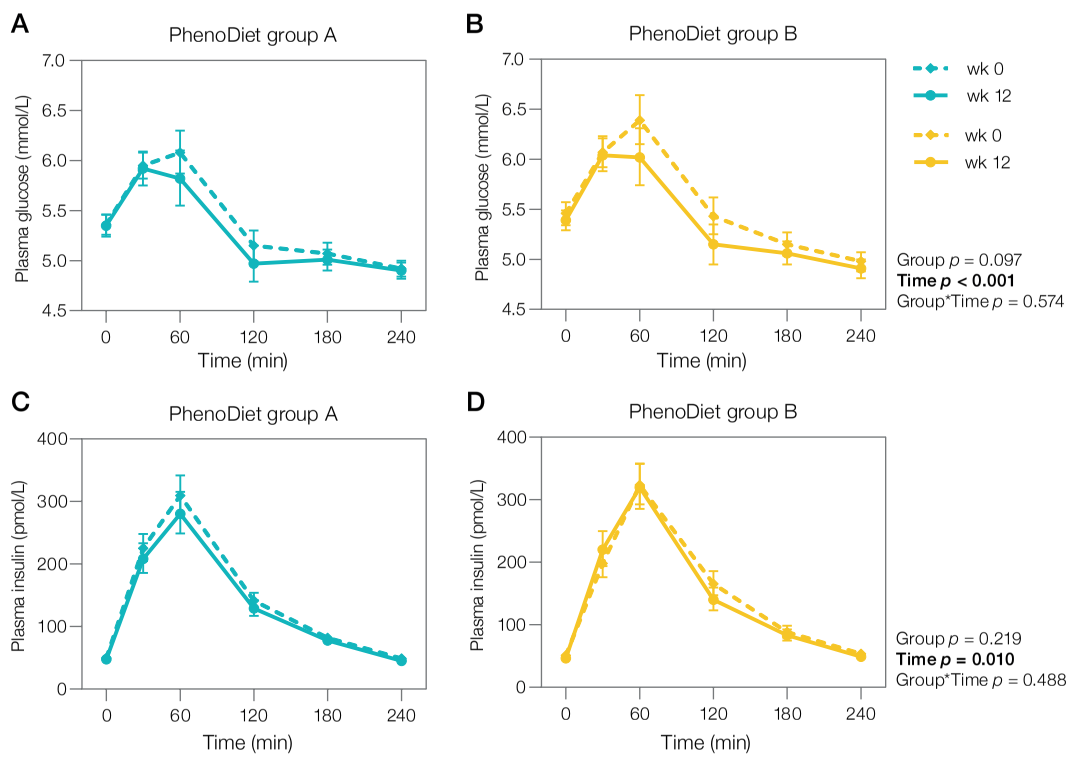

Fig. 3S. Plasma glucose and insulin responses to a liquid high-fat mixed meal, Related to Figure 2. Plasma glucose concentrations in PhenoDiet group A (A) and B (B), and plasma insulin concentrations in PhenoDiet group A (C) and B (D) in response to a liquid high-fat mixed meal at week 0 and week 12. Dashed lines indicate week 0, solid lines week 12. Data are presented as geometric means with 95% CI. The differences in area under the curves (AUCs) between PhenoDiet group A and B were assessed using a mixed model with repeated measures with adjustments for age, sex, and center. The AUCs for both postprandial glucose and insulin decreased in both groups (*P* for time all < 0.05) without differences between PhenoDiet group A and B.

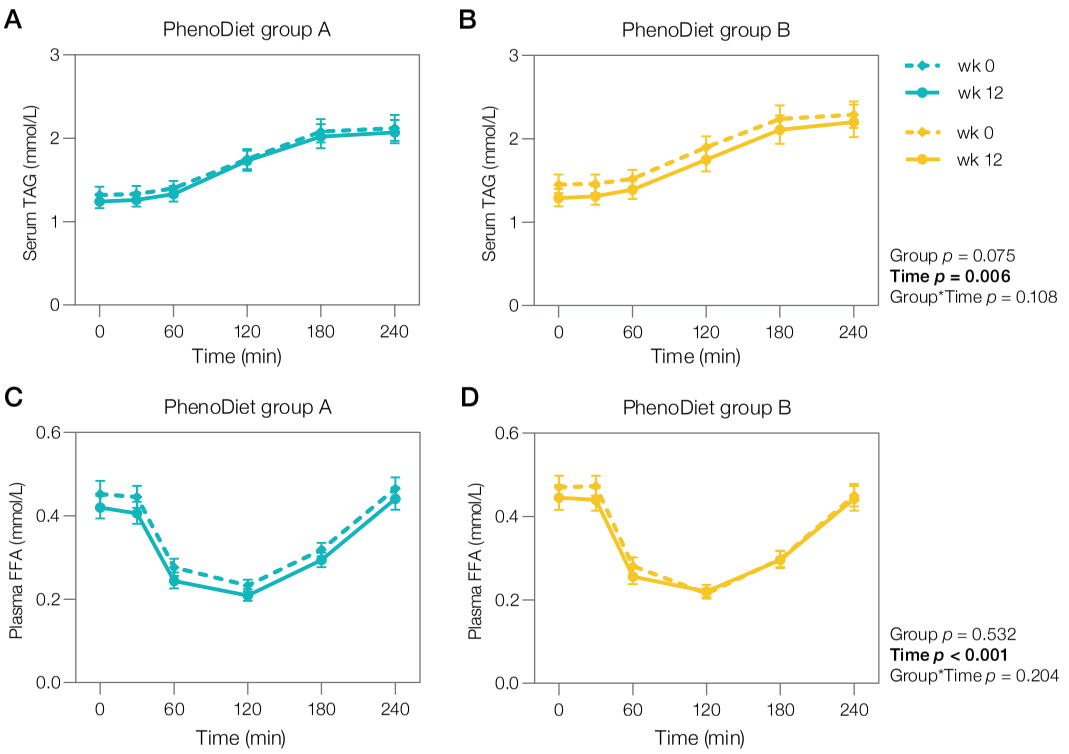

Fig. S4. Postprandial circulating TAG and FFA responses to a liquid high-fat mixed meal, Related to Figure 2. Serum triacylglycerol (TAG) concentrations in PhenoDiet group A (A) and B (B), and plasma free fatty acid (FFA) concentrations in PhenoDiet group A (C) and B (D) in response to a liquid high-fat mixed meal at week 0 and week 12. Dashed lines indicate week 0, solid lines week 12. Data are presented as geometric means with 95% CI. The differences in area under the curves (AUCs) between PhenoDiet group A and B were assessed using a mixed model with repeated measures with adjustments for age, sex, and center. The AUCs for both postprandial FFA and TAG decreased in both groups (*P* for time all < 0.05 are highlighted in bold. FFA, free fatty acids; TAG, triacylglycerol

Table S1. Targeted nutrient composition of the HMUFA and LFHP diet

	HMUFA	LFHP
Fat, (en%)	38	28
Monounsaturated fat	20	10
Polyunsaturated fat	8	8
Saturated fat	8	8
Protein (en%)	14	24
Animal-based (% of total protein)	45	60
Plant-based (% of total protein)	55	40
Carbohydrates (en%)	42	42
Mono- and disaccharides	12	12
Polysaccharides	30	30
Fiber (g/MJ)	3	>4
Alcohol	<3	<3

en%, energy percentage of total energy intake; MJ, megajoule

Table S2. Baseline characteristics with stratification for IR phenotype and diet intervention

	MIR phenotype			LIR phenotype			*P*-value	
	PhenoDiet group A	PhenoDiet group B	*P*-value	PhenoDiet group A	PhenoDiet group B	*P*-value		
	HMUFA diet	LFHP diet	HMUFA vs. LFHP	LFHP diet	HMUFA diet	HMUFA Vs. LFHP	MIR vs. LIR	HMUFA vs. LFHP
Age, years	60 ± 8	61 ± 8	0.752	60 ± 7	60 ± 8	0.972	0.596	0.808
Women, n (%)	45 (59.2%)	48 (65.8%)	0.499	21 (46.7%)	27 (56.3%)	0.409	0.109	1.000
BMI, kg/m^2	29.7 ± 3.6	29.3 ± 3.0	0.691	30.1 ± 1.3	30.7 ± 3.8	0.259	**0.037**	0.277
Medication use, n (%)								
Antidepressants	2 (2.6%)	8 (11.0%)	0.053	3 (6.7%)	4 (8.3%)	1.000	0.801	0.212
Antihypertensives	18 (23.7%)	12 (16.4%)	0.311	9 (20.0%)	4 (8.3%)	0.138	0.300	1.000
Anti-inflammatory	11 (14.5%)	8 (11.0%)	0.626	3 (6.7%)	1 (2.1%)	0.351	**0.041**	1.000
Statins	6 (7.9%)	5 (6.8%)	1.000	3 (6.7%)	2 (4.2%)	0.671	0.606	1.000
Other	27 (35.5%)	24 (32.9%)	0.863	15 (33.3%)	13 (27.1%)	0.652	0.574	1.000
Family history of DM, n (%)	15 (19.7%)	19 (26.0%)	0.436	7 (15.6%)	13 (27.1%)	0.212	0.875	1.000
Glucose status, n (%)			0.231			0.712	0.131	0.256
NGT	61 (80.3%)	52 (71.2%)		33 (73.3%)	36 (75.0%)			
IFG	2 (2.6%)	0 (0%)		3 (6.7%)	4 (8.3%)			
IGT	7 (9.2%)	13 (17.8%)		5 (11.1%)	3 (6.3%)			
Combined IFG/IGT	3 (3.9%)	2 (2.7%)		0 (0%)	2 (4.2%)			
T2DM	3 (3.9%)	6 (8.2%)		2 (4.4%)	3 (6.3%)			
Habitual physical activity, Baecke score	8.3 ± 1.2	8.3 ± 1.1	0.714	8.5 ± 1.3	8.3 ± 1.3	0.390	0.436	0.401
Employment status, n (%)			**0.009**			0.482	0.262	0.064
Paid job	45 (60.0%)	29 (39.7%)		28 (62.2%)	26 (54.2%)			
Retired	25 (33.3%)	28 (38.4%)		9 (20.0%)	15 (31.3%)			
Other	5 (6.7%)	16 (21.9%)		8 (17.8%)	7 (14.6%)			
Education level, n (%)			**0.034**			**0.045**	**0.035**	**0.002**
Low	3 (3.9%)	12 (16.4%)		15 (33.3%)	6 (12.5%)			
Intermediate	35 (46.1%)	27 (37.0%)		12 (26.7%)	21 (43.8%)			
High	37 (48.7%)	33 (45.2%)		18 (40.0%)	21 (43.8%)			

Values are n (%) or mean ± SD. *P*-values <0.05 are highlighted in bold

MIR, muscle insulin resistance; LIR, liver insulin resistance; BMI, body mass index; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.

Table S3. Baseline characteristics of drop-outs compared to completers

	PhenoDiet group A		PhenoDiet group B	
	Study completers (n = 114)	Drop-outs (n = 7)	Study completers (n = 107)	Drop-outs (n = 14)
Age, years	60 ± 7	54 ± 13	61 ± 8.2	59 ± 8
Women, n (%)	60 (52.6%)	6 (85.7%)	67 (62.6%)	8 (57.1%)
BMI, kg/m^2	29.7 ± 3.4	32.0 ± 5.6	29.9 ± 3.4	30.2 ± 3.1
Medication use, n (%)				
Antidepressants	4 (3.5%)	1 (14.3%)	11 (10.3%)	1 (7.1%)
Antihypertensives	25 (21.9%)	2 (28.6%)	15 (14.0%)	1 (7.1%)
Anti-inflammatory medication	14 (12.3%)	0 (0%)	8 (7.5%)	1 (7.1%)
Statins	9 (7.9%)	0 (0%)	7 (6.5%)	0 (0%)
Other	40 (35.1%)	2 (28.6%)	33 (30.8%)	4 (28.6%)
Family history of diabetes, n (%)	19 (16.7%)	3 (42.9%)	29 (27.1%)	3 (21.4%)
Glucose status, n (%)				
NGT	89 (78.1%)	5 (100%)	78 (72.9%)	10 (71.4%)
IFG	5 (4.4%)	0 (0%)	4 (3.7%)	0 (0%)
IGT	12 (10.5%)	0 (0%)	15 (14.0%)	1 (7.1%)
Combined IFG/IGT	3 (2.6%)	0 (0%)	2 (1.9%)	2 (14.3%)
T2D	5 (4.4%)	0 (0%)	8 (7.5%)	1 (7.1%)
Habitual physical activity, Baecke score	8.4 ± 1.2	8.3 ± 1.3	8.3 ± 1.2	8.4 ± 1.1
Employment status, n (%)				
Paid job	68 (59.6%)	5 (71.4%)	47 (43.9%)	8 (57.1%)
Retired	33 (28.9%)	1 (14.3%)	39 (36.4%)	4 (28.6%)
Other	12 (10.5%)	1 (14.3%)	21 (19.6%)	2 (14.3%)
Education level, n (%)				
Low	17 (14.9%)	1 (14.3%)	16 (15.1%)	2 (14.3%)
Intermediate	42 (36.8%)	5 (71.4%)	43 (40.6%)	5 (35.7%)
High	54 (47.4%)	1 (14.3%)	47 (44.3%)	7 (50.0%)

Values are n (%) or mean ± SD.
MIR, muscle insulin resistance; LIR, liver insulin resistance; BMI, body mass index; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes.

Table S4. Habitual dietary intake at baseline as assessed by FFQ

	PhenoDiet group A (n = 104)	PhenoDiet group B (n = 109)
Energy (MJ)	9.6 [7.8, 10.9]	8.6 [7.4, 10.6]
Fat (en%)	37.9 ± 5.9	37.5 ± 5.5
Monounsaturated fat	13.6 ± 2.8	13.4 ± 2.2
Polyunsaturated fat	7.3 ± 1.8	7.2 ± 1.8
Saturated fat	13.8 ± 2.5	13.8 ± 2.9
Protein (en%)	15.6 ± 2.0	15.6 ± 2.1
Animal-based (% of total protein)	58.9 ± 9.3	59.2 ± 8.2
Plant-based (% of total protein)	41.1 ± 9.3	40.9 ± 8.2
Carbohydrates (en%)	41.1 ± 5.9	41.9 ± 5.6
Mono- and disaccharides	19.0 ± 5.4	19.0 ± 5.2
Polysaccharides	22.1 ± 4.7	22.8 ± 4.5
Fiber (g/MJ)	2.6 ± 0.6	2.6 ± 0.6
Alcohol	2.2 [0.6, 4.0]	1.5 [0.6, 3.5]

Values are mean ± SD or median [IQR] if not normally distributed.
FFQ, food frequency questionnaire; MJ, megajoule; en%, energy percentage.

Table S5. Advised macronutrient composition of the intervention diets and reported intake

	Advised		PhenoDiet group A		PhenoDiet group B		*P*-value
	HMUFA	LFHP	MIR – HMUFA (n = 62)	LIR – LFHP (n = 41)	LIR – HMUFA (n = 42)	MIR - LFHP (n = 61)	HMUFA vs. LFHP
Energy (MJ)	9.8 ± 1.6	9.5 ± 1.6	8.1 ± 1.4	8.6 ± 1.7	8.6 ± 2	8.1 ± 1.4	0.966
Fat (en%)	38.3 ± 0.4	27.5 ± 0.5	34.8 ± 5.1	27.1 ± 3.3	33.9 ± 5.4	28.3 ± 4.4	**<0.001**
Monounsaturated fat	19.8 ± 0.5	9.7 ± 0.1	16.5 ± 3.7	9.2 ± 1.6	16.0 ± 3.7	9.4 ± 1.7	**<0.001**
Polyunsaturated fat	8.1 ± 0.3	7.7 ± 0.2	7.4 ± 1.7	6.6 ± 1.5	7.2 ± 1.4	7.4 ± 1.8	0.365
Saturated fat	7.8 ± 0.2	7.3 ± 0.2	8.4 ± 1.3	8.7 ± 1.7	8.3 ± 1.1	8.8 ± 1.8	0.071
Protein (en%)	14.0 ± 0.2	23.5 ± 0.2	16.6 ± 2.2	24.5 ± 2.1	17.3 ± 2.5	22.9 ± 3.2	**<0.001**
Animal-based, % of total	45.1 ± 1.3	60.1 ± 0.6	50.9 ± 9.2	65.7 ± 5.4	52.2 ± 7.8	60.9 ± 8.9	**<0.001**
Plant-based, % of total	54.9 ± 1.3	39.9 ± 0.6	49.1 ± 9.2	34.4 ± 5.4	47.8 ± 7.8	39.1 ± 8.9	**<0.001**
Carbohydrates (en%)	41.4 ± 0.4	42.4 ± 0.5	43.8 ± 4.8	43.4 ± 4.4	44.0 ± 4.5	43.6 ± 4.2	0.538
Mono- and disaccharides	12.9 ± 0.5	13.4 ± 0.3	15.6 ± 3.6	16.4 ± 2.7	15.2 ± 2.5	15.6 ± 3	0.204
Polysaccharides	28.5 ± 0.8	29.0 ± 0.5	28.3 ± 4.4	27.0 ± 3.5	28.8 ± 3.6	28.0 ± 3.3	0.084
Fiber (g/MJ)	3.0 ± 0.1	4.4 ± 0.2	3.3 ± 0.5	4.0 ± 0.6	3.3 ± 0.5	4.0 ± 0.6	**<0.001**
Alcohol	3.3 ± 0.4	2.4 ± 0.3	1.5 ± 2.2	0.9 ± 1.7	1.5 ± 1.9	1.3 ± 2.2	0.166

Values are mean ± SD. Advised intake is based on the targeted macronutrient composition (Supplementary Table 1) according to the individual meal plans as described in more detail elsewhere (24). *P*-values <0.05 are highlighted in bold.
MIR, muscle insulin resistance; LIR, liver insulin resistance; en%, energy percentage of total energy intake; MJ, megajoule.

Table S6. Effects of 12-week HMUFA and LFHP diet on plasma FA concentrations

	HMUFA		LFHP		*P*-value		
	Week 0	Week 12	Week 0	Week 12	Diet	Time	Diet x Time
Total FA (mmol/L)	13.2 (12.7 - 13.6)	12.5 (12.1 - 12.9)	13.4 (12.9 - 13.8)	12.4 (11.9 - 12.8)	0.918	**<0.001**	0.117
MUFA (mmol/L)	3.4 (3.2 - 3.5)	3.3 (3.2 - 3.4)	3.5 (3.3 - 3.7)	3.2 (3.0 - 3.3)	0.977	**<0.001**	**0.001**
MUFA (%)	25.7 (25.3 - 26)	26.4 (26.0 - 26.8)	26.2 (25.8 - 26.6)	25.8 (25.4 - 26.2)	0.891	0.074	**<0.001**
PUFA (mmol/L)	5.4 (5.3 - 5.5)	5.1 (5.0 - 5.3)	5.4 (5.3 - 5.6)	5.1 (5.0 - 5.3)	0.861	**<0.001**	0.902
PUFA (%)	41.0 (40.6 - 41.5)	41.0 (40.6 - 41.5)	40.6 (40.1 - 41.1)	41.6 (41.1 - 42.0)	0.879	**<0.001**	**<0.001**
PUFA/MUFA ratio	1.60 (1.56 - 1.64)	1.56 (1.52 - 1.59)	1.55 (1.51 - 1.59)	1.61 (1.57 - 1.65)	0.888	0.467	**<0.001**
SFA (mmol/L)	4.4 (4.2 - 4.5)	4.0 (3.9 - 4.2)	4.4 (4.2 - 4.6)	4.0 (3.9 - 4.2)	0.927	**<0.001**	0.261
SFA (%)	33.1 (32.9 - 33.3)	32.4 (32.2 - 32.7)	33.0 (32.8 - 33.3)	32.5 (32.2 - 32.7)	0.990	**<0.001**	0.364

Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center.

Table S7. Post-hoc analyses of intervention effects on secondary outcomes with stratification for IR phenotype

	MIR phenotype		LIR phenotype	
	PhenoDiet group A	PhenoDiet group B	PhenoDiet group A	PhenoDiet group B
	HMUFA	LFHP	LFHP	HMUFA
Fasting insulin (pmol/L)				
Week 0	47.5 (42.9 - 52.6)	49.9 (45.1 - 55.3)	47.5 (41.8 - 53.9)	57.0 (50.7 - 64.2)
Week 12	47.0 (42.6 - 51.8)	43.7 (39.5 - 48.3)	44.4 (38.5 - 51.2)	49.4 (43.1 - 56.5)
P-value time	0.762	**< 0.001**	0.230	**0.008**
2-hr glucose (mmol/L)				
Week 0	6.5 (6.1 - 6.9)	6.8 (6.4 - 7.3)	5.5 (5.0 - 6.1)	6.1 (5.5 - 6.7)
Week 12	6.4 (6.0 - 6.9)	6.4 (6.0 - 6.9)	5.8 (5.3 - 6.4)	5.8 (5.3 - 6.3)
P-value time	0.714	**0.034**	0.193	0.123
2-hr insulin (pmol/L)				
Week 0	370.8 (316.7 - 434.2)	429.5 (366.9 - 502.8)	295.9 (233.3 - 375.2)	342.8 (274.6 - 427.8)
Week 12	362.7 (310.8 - 423.2)	343.3 (292.9 - 402.3)	285.0 (230.7 - 352.2)	291.1 (238.1 - 355.8)
P-value time	0.722	**< 0.001**	0.688	0.069
HOMA-IR (AU)				
Week 0	1.6 (1.4 - 1.8)	1.7 (1.5 - 1.9)	1.6 (1.4 - 1.9)	2.0 (1.8 - 2.3)
Week 12	1.6 (1.4 - 1.8)	1.5 (1.3 - 1.7)	1.5 (1.3 - 1.8)	1.7 (1.5 - 2.0)
P-value time	0.481	**< 0.001**	0.274	**0.003**
Matsuda index (AU)				
Week 0	5.0 (4.5 - 5.6)	4.5 (4.0 - 5.0)	4.6 (4.0 - 5.4)	3.9 (3.4 - 4.6)
Week 12	5.1 (4.5 - 5.7)	5.4 (4.8 - 6.1)	5.1 (4.4 - 6)	4.6 (4.0 - 5.3)
P-value time	0.697	**< 0.001**	0.099	**0.008**
MISI (AU)				
Week 0	0.114 (0.098 - 0.132)	0.119 (0.102 - 0.139)	0.146 (0.118 - 0.181)	0.112 (0.092 - 0.137)
Week 12	0.112 (0.094 - 0.133)	0.147 (0.123 - 0.176)	0.162 (0.136 - 0.194)	0.156 (0.131 - 0.185)
P-value time	0.887	**0.030**	0.388	**0.007**
Serum TAG (mmol/L)				
Week 0	1.2 (1.1 - 1.4)	1.5 (1.4 - 1.6)	1.4 (1.2 - 1.6)	1.4 (1.3 - 1.6)
Week 12	1.2 (1.1 - 1.4)	1.3 (1.2 - 1.4)	1.3 (1.1 - 1.4)	1.3 (1.1 - 1.4)
P-value time	0.894	**< 0.001**	**0.019**	**0.006**
Plasma CRP (mg/L)				
Week 0	1.1 (0.9 - 1.4)	1.3 (1.0 - 1.6)	0.8 (0.6 - 1.1)	0.9 (0.7 - 1.2)
Week 12	1.2 (0.9 - 1.6)	0.9 (0.7 - 1.2)	0.7 (0.5 - 1.0)	0.8 (0.6 - 1.1)
P-value time	0.727	**0.005**	0.433	0.240

Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center. *P*-values <0.05 are highlighted in bold.
MIR, muscle insulin resistance; LIR, liver insulin resistance; HOMA-IR, homeostasis model assessment of insulin resistance; MISI, muscle insulin sensitivity index; TAG, triacylglycerol; CRP, C-reactive protein

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Tissue-specific insulin resistance phenotype-diet interactions in fasting and postprandial metabolite responses to a 12-week high-MUFA or low-fat, high-protein, high-fibre diet: a secondary analysis of the PERSON study

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In preparation

Abstract

Background

We previously showed that modulation of dietary macronutrient composition according to an individual's tissue-specific insulin resistance (IR) phenotype resulted in greater improvements in cardiometabolic health: individuals with predominant muscle IR (MIR) benefitted more from a diet low in fat and rich in protein and fibre (LFHP), while individuals with predominant liver IR (LIR) benefitted more from a diet rich in mono-unsaturated fat (HMUFA).

Objective

We investigated the effects of this dietary intervention on fasting and postprandial plasma metabolite profile to further characterise the effects of LFHP and HMUFA diets and their interaction with tissue-specific IR.

Methods

We performed a secondary analysis of the PERSON study, a two-centre, randomised, double-blind, dietary intervention trial in which 242 individuals with MIR or LIR (40-75 years, BMI 25-40 kg/m^2) were randomised to follow a HMUFA or LFHP diet for 12 weeks. Before and after the intervention, plasma samples were collected before (T=0) and after (T=30, 60, 120, 240 min) consumption of a high-fat mixed meal for quantification of 247 metabolite measures including lipoproteins, apolipoproteins, cholesterol, triglycerides (TAG), ketone bodies, and amino acids using nuclear magnetic resonance spectroscopy.

Results

A larger reduction in fasting VLDL-TAG and VLDL particle size was observed in individuals with MIR following the LFHP diet and those with LIR following the HMUFA diet. No IR phenotype-diet interactions were found for postprandial plasma metabolites. Irrespective of IR phenotype, the LFHP diet induced greater reductions in fasting and postprandial plasma concentrations of almost all subclasses of VLDL particles, small HDL particles, TAG fractions in most VLDL subclasses and the smaller LDL and HDL subclasses, β-hydroxybutyrate, and in postprandial branched-chain amino acid concentrations.

Conclusions

Precision nutrition according to IR phenotype enhanced improvements in fasting plasma VLDL profile, but overall, a LFHP diet may be more effective than a HMUFA diet for improving fasting and postprandial plasma metabolite profile and thereby reduce cardiometabolic health risk in individuals with tissue-specific IR, irrespective of IR phenotype.

Introduction

To maintain metabolic homeostasis in varying circumstances, humans have evolved a sophisticated system to efficiently store nutrients during feeding and mobilise stored nutrients during fasting. The chronic energy and nutrient oversupply of modern times puts a major strain on this system and can disrupt metabolic processes in the key organs that orchestrate metabolic regulation, i.e. the liver, adipose tissue, skeletal muscle, and pancreas.[1] These metabolic perturbations can result in insulin resistance, dyslipidemia, ectopic fat accumulation, and low-grade systemic inflammation, which in turn increase the risk of cardiometabolic diseases such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).[2,3] The development of cardiometabolic disease is characterised by great heterogeneity, with large inter-individual differences in both the aetiology of metabolic abnormalities and trajectories towards overt cardio-metabolic disease.[4-10]

Improving diet quality is an important strategy for the amelioration and prevention of cardiometabolic complications. The health effects of nutritional interventions are commonly assessed by measuring classical clinical biomarkers of metabolic health, such as fasting glucose, triglycerides, and cholesterol. While assessing change in such measures is useful in the clinic to estimate disease risk reduction, these measures fail to capture more subtle metabolic changes and provide little insight into underlying physiology. Extensive profiling of circulating metabolites, i.e. the use of metabolomics, allows for a more comprehensive investigation of diet effects on (patho)physiological processes in the liver, adipose tissue, and skeletal muscle.[11] In addition, metabolite changes upon a meal challenge may provide more insights into the functioning of these key metabolic organs than fasting metabolite levels because they reflect the metabolite production, secretion, and clearance capacity of the various metabolic organs. The postprandial response to a dietary stressor such as a lipid- or carbohydrate-rich meal has been proposed to be a particularly sensitive measure of metabolic health because it reveals how well the body can cope with a metabolic challenge and regain homeostasis.[12]

Although there is broad consensus on the main ingredients of a healthy dietary pattern for improving metabolic health,[13] the exact diet composition that is most optimal may differ per individual given the metabolic heterogeneity between individuals. Indeed, metabolic phenotype, as characterised by plasma glucose and insulin concentrations or indices based on these concentrations, has been recognised to modify the response to dietary intervention.[14-18] We recently demonstrated that individuals with different tissue-specific insulin resistance (IR) phenotypes respond differentially to dietary macronutrient modulation with respect to effects on cardio-metabolic health. More specifically, individuals with predominant muscle IR (MIR) had greater improvements in whole-body insulin sensitivity, serum triglyceride (TAG)

levels, and serum C-reactive protein (CRP) on a diet low in fat and rich in protein and fibre (LFHP), while individuals with predominant liver IR (LIR) benefitted more from a diet rich in mono-unsaturated fat (HMUFA) with respect to these parameters.[19]

Here, we investigated the effects of these 12-week HMUFA and LFHP diets in MIR and LIR individuals on the plasma metabolite profile in both the fasting state and in response to a high-fat mixed meal. We aimed to further characterise the effects of LFHP and HMUFA diets in tissue-specific IR and identify leads towards potential underlying mechanisms of the differential effects of HMUFA and LFHP diets in individuals with tissue-specific IR.

Methods

Study design and participants

The current study is a secondary analysis of a two-centre, 12-week, randomised, double-blind, parallel, dietary intervention trial conducted from May 2018 until November 2021 at Maastricht University Medical Center+ (MUMC+) and Wageningen University (WUR) in the Netherlands. Study design and methodology have been described previously.[20] Inclusion criteria were: age 40–75 years, BMI 25–40 kg/m^2, body weight stability for at least three months (no weight gain or loss >3 kg), and tissue-specific IR, characterised as predominant LIR or MIR. Exclusion criteria included pre-diagnosis of type 2 diabetes, diseases or medication use that affect glucose or lipid metabolism, major gastrointestinal disorders, history of major abdominal surgery, uncontrolled hypertension, smoking, alcohol consumption >14 units/wk, and >4 h/wk moderate-to-vigorous physical activity. We additionally excluded statin users from the current analysis due to statins' effects on fasting and postprandial cholesterol and triglycerides.[21,22] Tissue-specific IR was assessed at screening based on the plasma glucose and insulin concentrations during a 7-point OGTT, from which the muscle insulin sensitivity index (MISI) and hepatic insulin resistance index (HIRI) were calculated.[23,24] Tertile cut-offs for MISI and HIRI from a previous study with a similar study population[25,26] were used to identify individuals with predominant MIR or LIR.

Dietary intervention

In total, 242 participants were included and randomly assigned to either Phenotype Diet (PhenoDiet) group A or PhenoDiet group B. PhenoDiet group A included individuals with MIR following a high-monounsaturated fatty acid diet (HMUFA) and individuals with LIR following a low-fat, high-protein, high-fibre diet (LFHP). PhenoDiet group B included individuals with MIR and LIR on LFHP and HMUFA diets, respectively. Both researchers and participants were blinded to the participants' metabolic phenotype (LIR or MIR), and thus blinded to whether participants were allocated to PhenoDiet A or B. The primary outcome of the original study was the change in disposition index, a composite marker of insulin secretion and insulin sensitivity, in PhenoDiet group A versus B.

The HMUFA diet had a targeted macronutrient composition of 38% of energy (en%) from fat (20 en% MUFA, 8 en% PUFA, 8 en% SFA), 48 en% carbohydrates (30 en% polysaccharides; 3 g/MJ fibre), and 14 en% protein. The macronutrient composition of the LFHP diet was targeted to be 28 en% fat (10 en% MUFA, 8 en% PUFA, 8 en% SFA), 48 en% carbohydrates (30 en% polysaccharides; >4 g/MJ fibre), and 24 en% protein. The reported macronutrient composition of the HMUFA and LFHP diets is described elsewhere.[19] Both diets were in line with the Dutch dietary guidelines.[27] Participants received key food products in pre-measured quantities and had weekly individual counselling sessions with a dietician or research nutritionist to monitor adherence to the diet and body weight stability. In case of weight loss or gain, the advised energy intake was adjusted to prevent further weight change. Details of the dietary intervention strategy have been reported previously.[20]

High-fat mixed-meal test

Before and after the intervention, participants visited the facilities after a 12-hour overnight fast for a high-fat mixed-meal test. The evening before this visit, participants consumed a standardised low-fat pasta meal (30% of energy intake [en%] fat, 49 en% CHO, 21 en% protein; 1,560–2,460 kJ, depending on estimated energy requirements), and they were instructed to refrain from alcohol and vigorous physical activities for three days before the visit. The liquid high-fat mixed meal was prepared in the metabolic kitchen using ice cream, full-fat milk, whipped cream, and sugar and contained 2.8 MJ, 49 g fat, 48 g carbohydrates, and 12 g protein (Table 1).

An intravenous cannula was inserted in the antecubital vein, and fasting blood samples were drawn at least 30 minutes after insertion. Participants consumed the meal within five minutes. Postprandial blood samples were drawn at t = 30, 60, 90, 120, 180, and 240 minutes.

Glucose and insulin levels were measured in EDTA plasma from timepoints 0, 30, 60, 120, 180, and 240 min by enzymatic assay or ELISA, respectively. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as *(fasting glucose in mmol/L × fasting insulin in mU/L) ÷ 22.5*. Fasting serum TAG, total cholesterol, and HDL cholesterol were quantified with enzymatic assays. Hypercholesterolemia was defined as fasting serum total cholesterol ≥5.0 mmol/L and hypertriglyceridemia as fasting serum TAG ≥1.7 mmol/L.

Fasting and postprandial plasma metabolite profile

Metabolite concentrations were quantified in EDTA plasma samples from T = 0, 30,

Table 1. Nutrient composition of the high-fat mixed meal

	Ice cream	Full-fat milk	Whipped cream	Sugar	Total per meal
Amount per meal, g	150	125	70	5	350
Energy, kJ	1388	348	973	85	2793
Protein, g	5.6	4.5	1.5	0	11.6
Fat, g	19.5	4.5	24.6	0	48.6
Saturated fat, g	12.8	3.1	17.5	0	33.4
Carbohydrates, g	34.5	5.9	2.2	5.0	47.5
Sugar, g	31.5	5.9	2.2	5.0	44.5

60, 120, and 240 min by the Nightingale high-throughput nuclear magnetic resonance (NMR) metabolomics platform (Nightingale Health Ltd., Helsinki, Finland)[28,29]. This platform provides quantitative data on 164 metabolites, including 14 lipoprotein subclasses (different sizes/subclasses of VLDL, IDL, LDL, and HDL), their lipid concentrations and composition, apolipoprotein A-I and B, major fatty acids, (branched-chain) amino acids, glycolysis-related measures, and ketone bodies. In addition, it provides data on the three lipoprotein sizes (VLDL, LDL, and HDL diameter) and 82 relative measures (i.e. percentages, ratios). We used clinically measured plasma glucose instead of NMR-measured glucose and excluded the measure 'Unsaturation', assessing a total of 247 metabolic measures.

Calculations

The postprandial total area under the curve (AUC) and net incremental area under the curve (iAUC) were calculated using the trapezoid method.[30] For calculation of (i) AUCs, metabolite curves from participants were excluded if values of ≥2 timepoints were missing (n = 5) and/or if the last (t = 240 min) value was missing (n = 7). For metabolite curves with one missing value at 30-120 minutes, the missing values were imputed with the weighted metabolite average of the two closest time points of that particular metabolite of that participant (n = 26).

Statistical analyses

Fasting metabolite concentrations and AUCs were log-transformed (log2) to obtain normal distributions and autoscaled to allow for a direct comparison of effect sizes. Intervention effects comparing PhenoDiet groups A and B or the two diets were tested using a linear mixed model with fasting plasma metabolite concentration or postprandial plasma metabolite AUC as a dependent variable; PhenoDiet or diet as a fixed factor; time as a repeated measure (baseline and week 12); and age, sex, and centre as covariates. For significant intervention effects on postprandial metabolites (AUC), we additionally tested intervention effects on metabolite iAUC to assess effects independent of changes in fasting metabolite concentrations. Data are reported as estimated marginal means with 95% confidence intervals.

Many of the 247 metabolite measures investigated in this study are highly correlated. Therefore, we estimated the number of independent tests performed by calculating how many principal components explained 95% of the variation in the data. Statistical significance after adjustment for multiple testing was set at $p < 0.00139$ (i.e., 0.05/19/2) to account for performing two sets of analyses (fasting and postprandial metabolites) in 19 independent measures. However, due to the exploratory nature of this study, we also describe intervention effects with a two-tailed $p < 0.05$. Analyses were performed using IBM SPSS Statistics software version 28.

Results

Two hundred twenty-one participants completed the study, of which 22 completed the study according to an adjusted protocol employed during the COVID-19 lockdown and did not undergo a high-fat mixed-meal test after 12 weeks of intervention. After the exclusion of statin users, plasma metabolite data were available from 214 participants at baseline and 179 participants post-intervention. The participants' mean (± SD) age was 60 ± 8 years, and 61% were women (Table 2). Sixty-seven per cent of individuals with MIR and 75% of individuals with LIR had a fasting serum cholesterol level ≥5.0 mmol/L. Twenty-eight per cent of individuals with MIR and 36% of individuals with LIR had a fasting serum triglyceride level ≥1.7 mmol/L.

Fasting plasma metabolite profile

PhenoDiet groups A versus B

We first explored whether fasting plasma metabolites were differentially affected in PhenoDiet groups A versus B. Fasting levels of 11 absolute metabolites, 20 relative metabolite measures, and one lipoprotein particle size were significantly differentially changed between PhenoDiet groups A and B (Fig. 1). Figures 2-4 show the standardised mean changes in lipoprotein particle concentrations, lipoprotein particle size, and the TAG fractions of the lipoprotein subclasses. Other results can be found in Table S1.

The majority of differences in the 11 absolute plasma metabolites were in the TAG fraction of VLDL particles. Plasma levels of total TAG, VLDL-TAG, and TAG in the small (S), medium (M), large (L), and very large (XL) VLDL subclasses were decreased

Table 2. Baseline characteristics of the study population according to insulin resistance phenotype and diet allocation

	PhenoDiet group A (n = 106)		PhenoDiet group B (n = 108)	
	MIR – HMUFA (n = 66)	LIR – LFHP (n = 40)	MIR – LFHP (n = 65)	LIR – HMUFA (n = 43)
Women, n (%)	40 (60.6%)	20 (50.0%)	44 (67.7%)	26 (60.5%)
Age, years	59.9 ± 8.1	59.0 ± 6.1	60.4 ± 8.5	59.1 ± 8.1
BMI, kg/m^2	29.7 ± 3.5	30.1 ± 3.5	29.5 ± 3.1	31.1 ± 4.1
Waist circumference, cm	100.9 ± 8.8	103.5 ± 9.8	101.0 ± 8.8	104.2 ± 11.7
Plasma glucose, mmol/L	5.3 ± 0.5	5.4 ± 0.6	5.3 ± 0.6	5.7 ± 0.8
Plasma insulin, pmol/L	45.7 [39.4, 58.7]	49.5 [41.0, 69.3]	42.8 [35.2, 61.8]	51.3 [41.0, 69.2]
HOMA-IR, AU	1.6 [1.3, 2.0]	1.8 [1.4, 2.4]	1.4 [1.2, 2.2]	1.8 [1.4, 2.7]
Serum total cholesterol, mmol/L	5.3 ± 0.9	5.4 ± 0.9	5.5 ± 1.0	5.6 ± 1.0
Serum HDL cholesterol, mmol/L	1.4 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3
Hypercholesterolemia, n (%)	42 (63.6%)	29 (72.5%)	46 (70.8%)	33 (76.7%)
Serum triglycerides, mmol/L	1.2 [1.0, 1.6]	1.4 [1.0, 1.8]	1.4 [1.1, 1.9]	1.5 [1.0, 1.9]
Hypertriglyceridemia, n (%)	15 (22.7%)	11 (27.5%)	22 (33.8%)	19 (44.2%)

Numerical data are presented as mean ± SD if normally distributed and as median [25th percentile, 75th percentile] if not normally distributed. Categorial data are presented as n (%).
BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; AU, arbitrary unit; HDL, high-density lipoprotein.

to a larger extent in PhenoDiet group B as compared to PhenoDiet group A (P for time < 0.001; P for group x time interaction < 0.05). Plasma concentrations of L VLDL particles were reduced in PhenoDiet group B, in line with a decrease in VLDL particle size, and not in PhenoDiet group A.

As for the relative metabolite measures, the main differences between PhenoDiet groups A and B were observed in the lipid composition of VLDL subclasses, which were in line with the above-described changes in absolute metabolite concentrations (Table S1).

None of the differential changes in fasting plasma metabolites between PhenoDiet groups A and B remained statistically significant after adjustment for multiple testing.

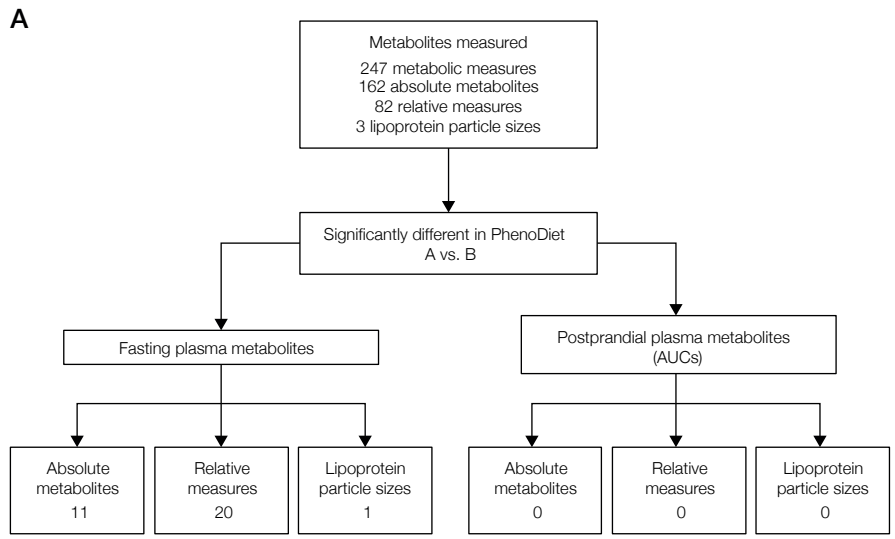

Figure 1. Flowchart of metabolites that were differentially affected in PhenoDiet group A versus B (A) and upon the LFHP vs. HMUFA diet (B). Intervention effects comparing PhenoDiet groups A and B or the two diets were tested using a linear mixed model with fasting plasma metabolite concentration or postprandial plasma metabolite AUC as a dependent variable, PhenoDiet or diet as a fixed factor, time as a repeated measure (baseline and week 12), and age, sex, and centre as covariates.

Diet effects

Next, we explored the effects of the LFHP and HMUFA diets on fasting and postprandial metabolite profiles regardless of IR phenotype. Changes in fasting levels of 31 absolute metabolites, 28 relative metabolite measures, and two lipoprotein particle sizes were significantly different between the diets (Fig. 1). Figures 2-4 show the standardised mean changes in lipoprotein particle concentrations, lipoprotein particle size, and the TAG fractions of the lipoprotein subclasses. Other results can be found in Table S2.

The majority of differences in the 31 absolute fasting plasma metabolite levels between the diets were found in the lipid fractions of HDL and VLDL particles and were caused mainly by a greater reduction upon the LFHP diet as compared to the HMUFA diet. The LFHP diet lowered particle concentrations of L and XL VLDL particles, while the HMUFA diet did not affect these particle concentrations (Fig. 2). The LFHP diet resulted in reductions in total TAG in VLDL, and TAG in S, L, and XL VLDL subclasses, as well as in total TAG in HDL, and TAG in the S and M HDL subclasses (Fig. 4). In contrast, the HMUFA diet did not affect these plasma metabolites. Both diets resulted in reductions in plasma particle concentrations of S HDL and its lipid components phospholipids, cholesterol, free cholesterol, and cholesteryl esters, but reductions were significantly larger upon the LFHP diet (P for time < 0.001; P for group x time interaction < 0.05) (Fig. 2, Table S2). In addition, the LFHP diet decreased VLDL size and increased LDL size, while the HMUFA diet did not affect these lipoprotein particle sizes (Fig. 3).

For fatty acids, total MUFA was reduced upon both diets, but to a larger extent upon the LFHP diet, while the PUFAs DHA and omega-3 FA were decreased upon the HMUFA diet, and not upon the LFHP diet (Table S2). Furthermore, β-hydroxybutyrate was reduced upon the LFHP diet and unaffected by the HMUFA diet (Fig. 3), and plasma citrate was increased upon the HMUFA diet and unaffected by the LFHP diet.

As for the relative fasting metabolite measures, the majority of the 28 differential changes between the LFHP and HMUFA diets concerned the lipid composition of lipoproteins and the composition of plasma FA, in line with the changes in absolute metabolite concentrations described above (Table S2).

After adjustment for multiple testing, changes in five metabolites remained statistically significantly different between the diets. These included the absolute amount of free cholesterol in XL HDL, which was increased after the LFHP diet and decreased upon the HMUFA diet, and the percentages of total MUFA, total PUFA, and omega-3 FA, as well as the ratio of PUFA to MUFA (Table S2).

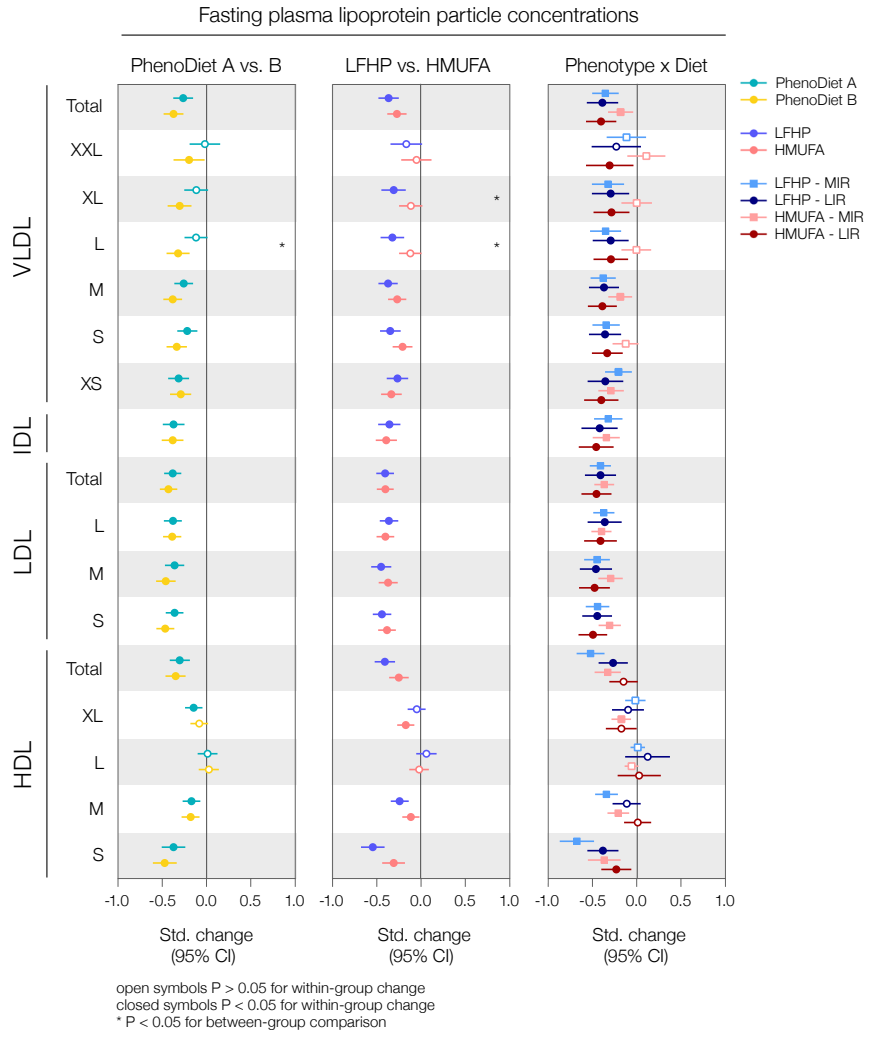

Figure 2. Dietary intervention effects on fasting plasma lipoprotein particle concentrations. Data are presented as standardised within-group mean changes with 95% CI. Effects on fasting metabolite concentrations between the PhenoDiet groups (left panel) and diets (middle panel) were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates. The right panel shows within-group changes according to IR phenotype and diet.

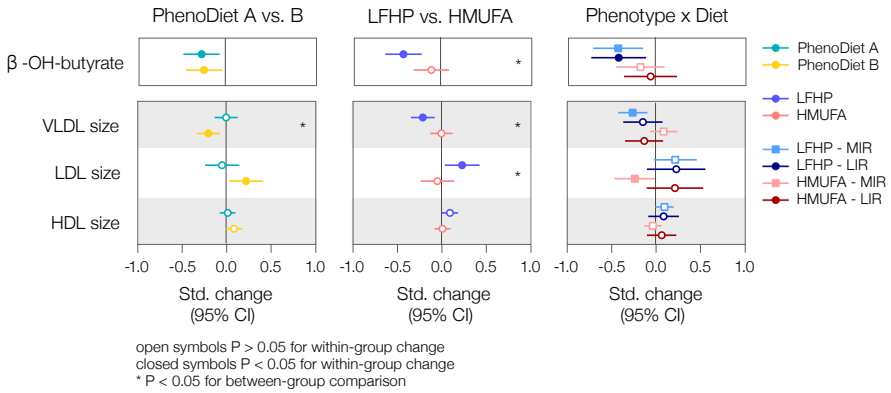

Figure 3. Dietary intervention effects on fasting β-hydroxybutyrate and lipoprotein particle sizes. Data are presented as standardised within-group mean changes with 95% CI. Effects on fasting metabolite concentrations between the PhenoDiet groups (left panel) and diets (middle panel) were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates. The right panel shows within-group changes according to IR phenotype and diet.

Postprandial plasma metabolite profiles

PhenoDiet groups A versus B

Changes in the postprandial AUCs of none of the measured metabolites differed significantly between PhenoDiet groups A and B (Fig. 1, Table S4).

Diet effects

Comparison of the effects of the LFHP and HMUFA diet resulted in significant differences in postprandial metabolites as determined by the AUCs of 66 absolute metabolites, 40 relative metabolite measures, and all three lipoprotein particle sizes (Fig. 1). The majority of the differences in postprandial plasma metabolite changes were in the particle concentrations and lipid content of HDL and VLDL particles, lipoprotein TAG fractions, FA, and BCAA. Overall, the LFHP diet resulted in greater reductions than the HMUFA diet. All significantly different postprandial metabolites between the diets can be found in Table S5.

The LFHP diet significantly reduced postprandial particle concentrations of all VLDL subclasses, M LDL, and S HDL (Fig. 5, Fig. S1). Furthermore, the LFHP diet lowered postprandial total TAG, and the TAG fraction of almost all VLDL subclasses, i.e. S-XL, S and M LDL, and all HDL subclasses, while the HMUFA diet only reduced TAG in M LDL, and to a smaller extent compared to the LFHP diet (Fig. 7, Fig. S1).

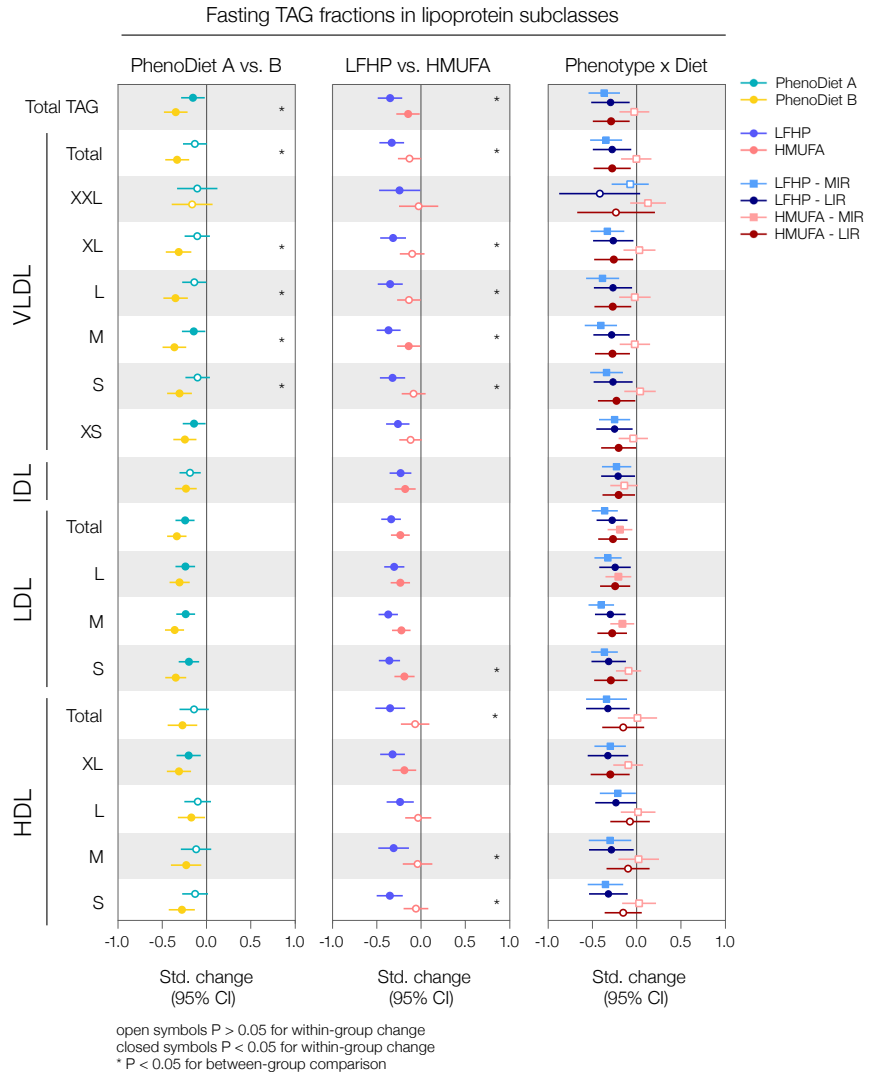

Figure 4. Dietary intervention effects on fasting TAG fractions in lipoprotein subclasses. Data are presented as standardised within-group mean changes with 95% CI. Effects on fasting metabolite concentrations between the PhenoDiet groups (left panel) and diets (middle panel) were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates. The right panel shows within-group changes according to IR phenotype and diet.

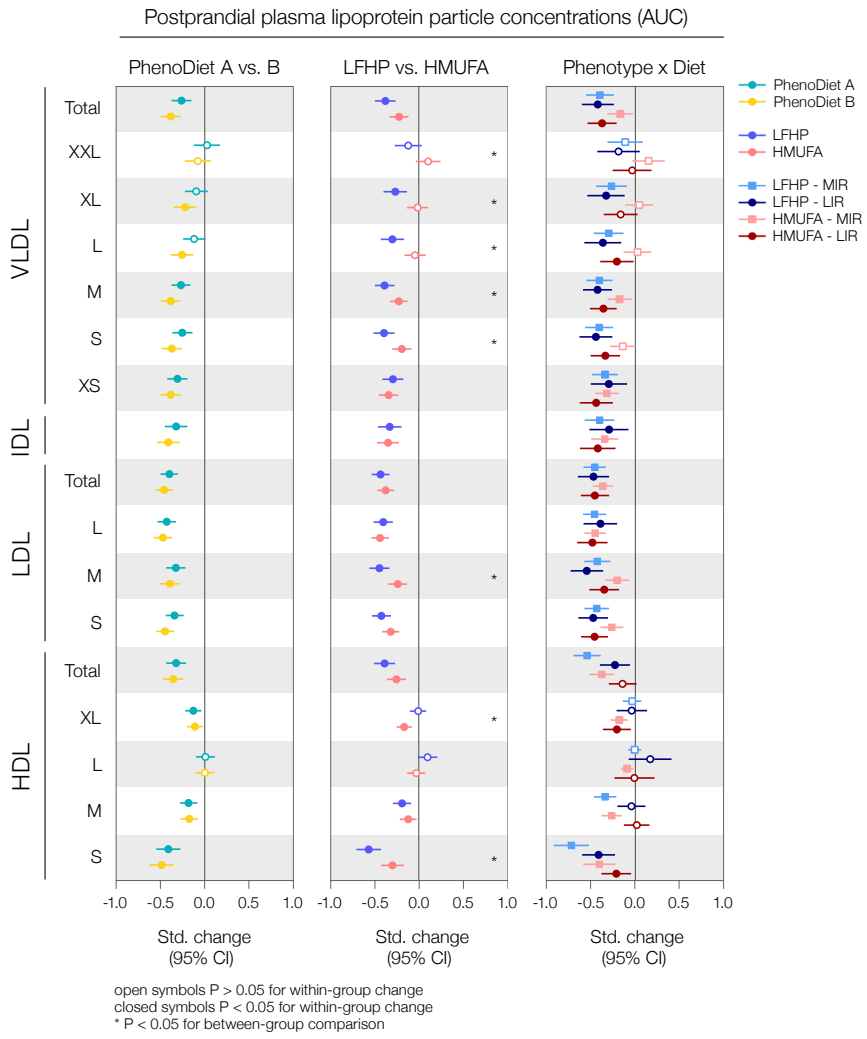

Figure 5. Dietary intervention effects on postprandial plasma lipoprotein particle concentrations. Data are presented as standardised within-group mean changes with 95% CI. Effects on postprandial metabolite concentrations (AUC) between the PhenoDiet groups (left panel) and diets (middle panel) were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates. The right panel shows within-group changes according to IR phenotype and diet.

The LFHP diet lowered postprandial VLDL size and increased postprandial HDL size, and the HMUFA diet increased postprandial VLDL size and decreased postprandial LDL size (Fig. 6).

As for the postprandial plasma FA concentrations, the LFHP diet lowered postprandial total MUFA, and the HMUFA diet decreased postprandial total omega-3 FA (Table S4). Furthermore, the LFHP diet reduced postprandial glutamine, isoleucine, leucine, and total BCAA levels, while the HMUFA diet increased postprandial glycine and did not affect postprandial glutamine or BCAA levels (Fig. 8, Fig. S1, Table S4). Both diets reduced postprandial β-hydroxybutyrate levels, but the LFHP diet resulted in larger reductions (Fig. 6, Fig. S1).

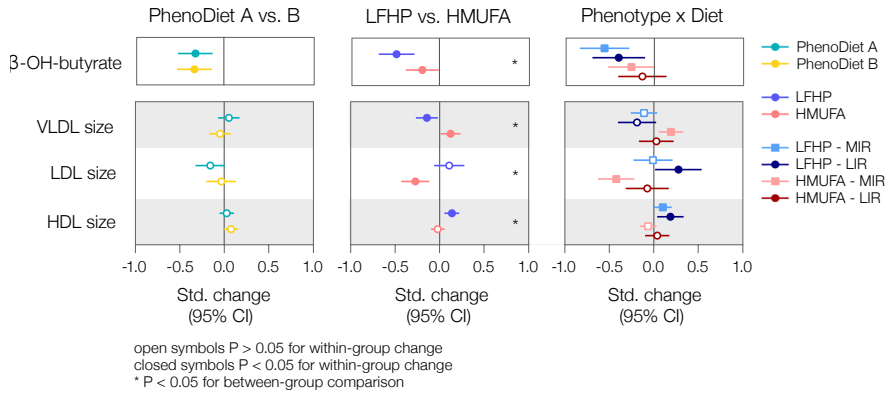

Figure 6. Dietary intervention effects on postprandial plasma β-hydroxybutyrate and lipoprotein particle sizes. Data are presented as standardised within-group mean changes with 95% CI. Effects on postprandial metabolite measures (AUC) between the PhenoDiet groups (left panel) and diets (middle panel) were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates. The right panel shows within-group changes according to IR phenotype and diet.

Only for TAG in S HDL, MUFA, the fatty acid ratios, as well as phospholipids, cholesterol, free cholesterol, and cholesteryl esters in XL HDL particles, differences remained statistically significant after adjustment for multiple testing (Table S4).

To examine whether the observed diet-induced changes in postprandial metabolites were due to a change in total postprandial exposure as determined with total AUC or due to a change in response, we additionally tested the effects of the two diets on the postprandial iAUCs (Table S5). The LFHP diet increased iAUCs of postprandial XL HDL particles and HDL size, while the HMUFA diet decreased iAUCs of phospholipids,

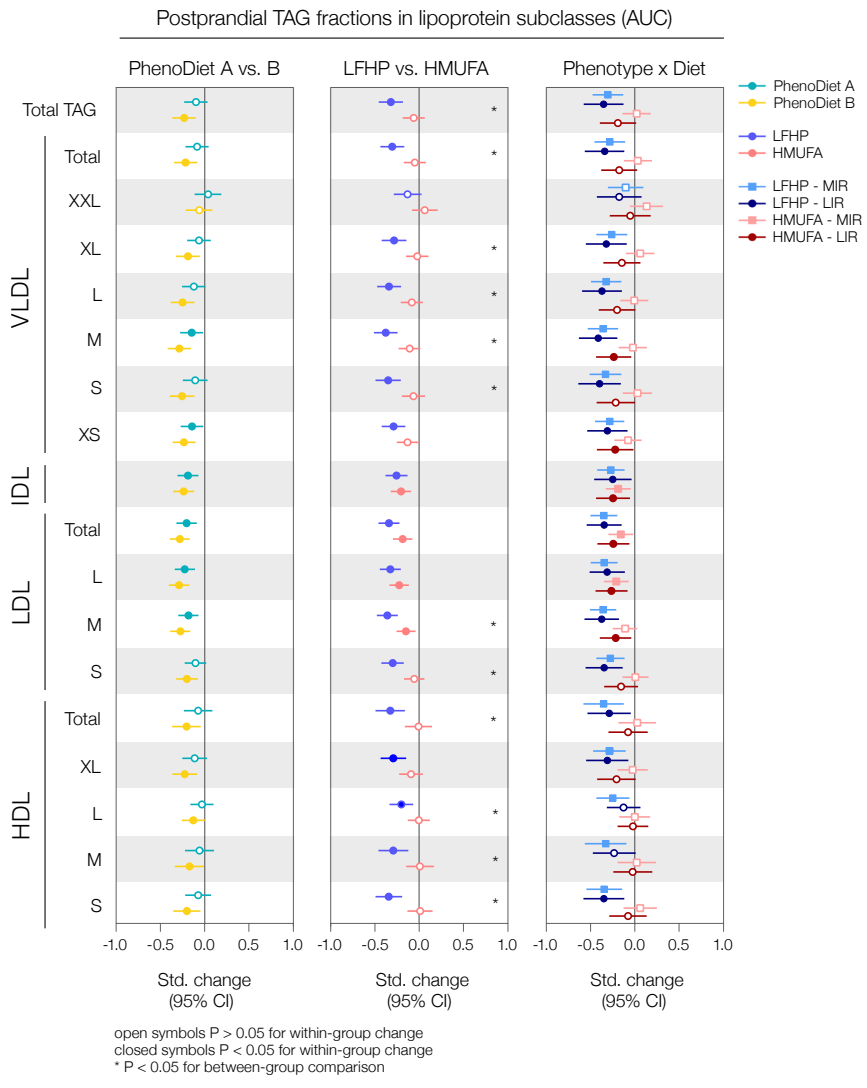

Figure 7. Dietary intervention effects on postprandial plasma triglycerides. Data are presented as standardised within-group mean changes with 95% CI. Effects on postprandial metabolite concentrations (AUC) between the PhenoDiet groups (left panel) and diets (middle panel) were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates. The right panel shows within-group changes according to IR phenotype and diet.

cholesterol and cholesteryl esters in XL HDL particles. The iAUC of postprandial glutamine was increased after the HMUFA diet and not changed after the LFHP diet. None of these effects remained statistically significant after adjustment for multiple testing.

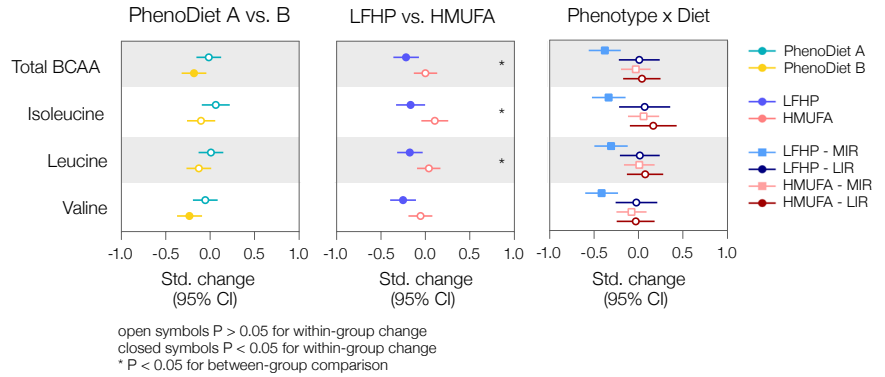

Figure 8. Dietary intervention effects on postprandial branched-chain amino acids. Data are presented as standardised within-group mean changes with 95% CI. Effects on postprandial metabolite concentrations (AUC) between the PhenoDiet groups (left panel) and diets (middle panel) were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates. The right panel shows within-group changes according to IR phenotype and diet.

Diet effects on glucose and insulin responses and cardiometabolic parameters

Because we found differential effects of the LFHP and HMUFA diet on postprandial plasma metabolites, we further examined the effects of these diets on plasma glucose and insulin responses upon the HFMM, as well as on other cardiometabolic parameters. Both diets lowered the plasma glucose and insulin responses upon the HFMM (Fig. 9). While reductions in postprandial insulin were similar upon both diets (P for time = 0.007; P for diet x time = 0.70), the HMUFA diet resulted in larger reductions in postprandial glucose compared to LFHP (P for diet x time = 0.005) (Fig. 9).

Compared to the HMUFA diet, the LFHP diet resulted in larger reductions in the android/gynoid ratio (P for time < 0.001; P for diet x time = 0.032), the insulinogenic index (P for time = 0.040; P for diet x time = 0.014), and a similar trend towards a larger reduction was observed for HIRI (P for time <0.001; P for diet x time = 0.050)

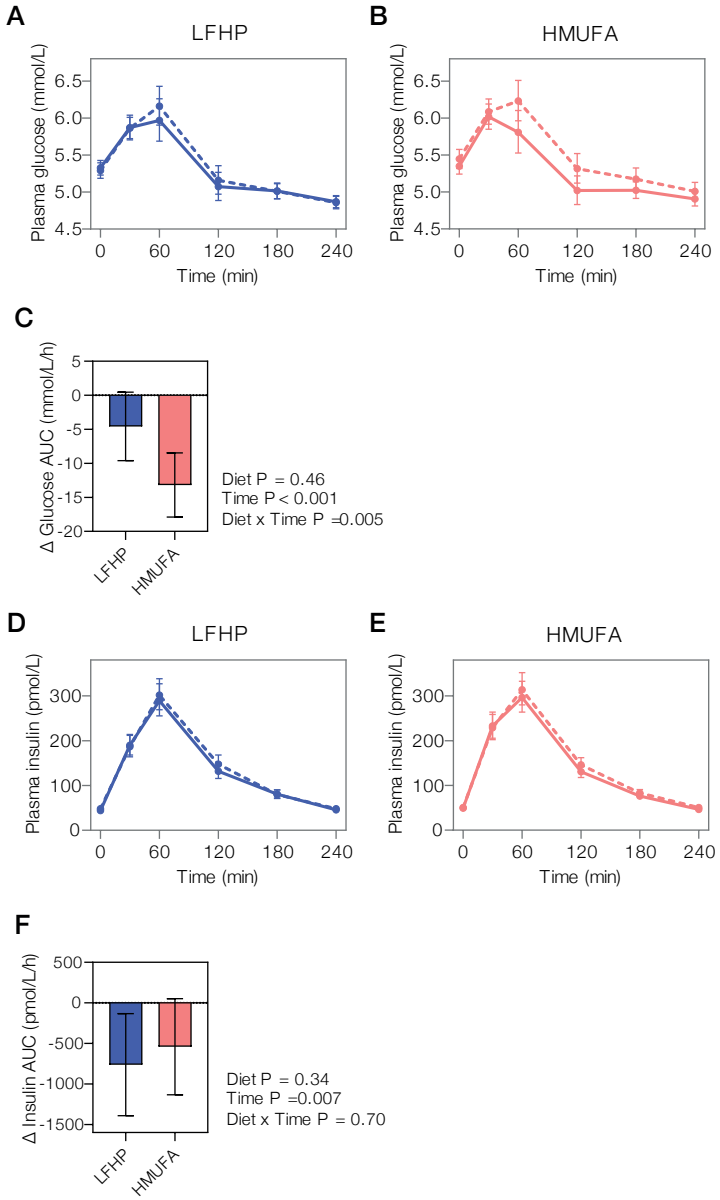

Figure 9. Plasma glucose (A-B) and insulin (D-E) responses to the high-fat mixed meal at week 0 (dotted lines) and week 12 (solid lines) upon the LFHP and HMUFA diet. Data are presented as geometric means with 95% CI. The differences in area under the curves (AUCs) between the diets were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates.

(Table 3). In addition, the LFHP diet also resulted in larger reductions in VAT, although this effect was only observed in the subgroup in which VAT was assessed using single-slice MRI (P for time < 0.001; P for diet x time = 0.041), and not in the subgroup in which VAT was assessed using a whole-body MRI (P for time < 0.001; P for diet x time = 0.24).

Table 3. Cardiometabolic parameters at baseline and after 12 weeks of LFHP or HMUFA diet

	LFHP diet (n = 108)		HMUFA diet (n = 116)		*P*-value		
	week 0	week 12	week 0	week 12	Diet	Time	Diet x Time
Anthropometrics							
Weight, kg	86.7 (84.7 - 88.7)	84.5 (82.6 - 86.5)	88.1 (86.3 - 90.2)	86.3 (84.3 - 88.3)	0.256	**<0.001**	0.283
Waist circumference, cm	101.6 (100 - 103.5)	99.3 (97.7 - 101.2)	101.9 (100.2 - 103.5)	100.2 (98.6 - 101.9)	0.714	**<0.001**	0.218
Waist-to-hip ratio	0.933 (0.923 - 0.946)	0.927 (0.916 - 0.938)	0.927 (0.916 - 0.938)	0.927 (0.918 - 0.938)	0.674	0.257	0.136
Body composition & ectopic fat							
Body fat mass, %	37.3 (36.5 - 38.3)	35.9 (34.9 - 36.9)	36.9 (36 - 37.8)	35.6 (34.6 - 36.6)	0.567	**<0.001**	0.565
Android fat mass, kg	3.2 (3.1 - 3.4)	2.9 (2.8 - 3.1)	3.3 (3.1 - 3.4)	3.1 (2.9 - 3.2)	0.326	**<0.001**	0.234
Gynoid fat mass, kg	5.2 (4.9 - 5.4)	4.9 (4.7 - 5.2)	5.0 (4.8 - 5.2)	4.7 (4.5 - 5)	0.333	**<0.001**	0.613
Android/gynoid ratio	1.17 (1.14 - 1.19)	1.14 (1.11 - 1.17)	1.20 (1.17 - 1.22)	1.20 (1.17 - 1.23)	**0.008**	**0.040**	**0.014**
Abdominal SAT, L[a]	10.5 (9.6 – 11.5)	9.9 (9.0 – 10.9)	9.7 (9.0 – 10.5)	9.2 (8.5 – 10.0)	0.214	**<0.001**	0.577
Abdominal SAT, cm^{2}[b]	256 (235 - 280)	232 (212 - 253)	291 (268 - 317)	271 (249 – 295)	**0.020**	**<0.001**	0.236
VAT, L[a]	5.5 (4.9 - 6.1)	5.1 (4.6 - 5.6)	5.1 (4.7 - 5.6)	4.8 (4.4 - 5.3)	0.429	**<0.001**	0.237
VAT, cm^{2}[b]	164 (151 - 177)	146 (134 - 158)	168 (156 - 182)	158 (146 - 172)	0.323	**<0.001**	**0.041**
Liver fat, %[a]	7.7 (5.8 - 10.1)	5.3 (4 - 7.1)	5.1 (4.1 - 6.5)	3.3 (2.6 - 4.2)	**0.017**	**<0.001**	0.388
Liver fat, %[b]	2.8 (2.1 - 3.8)	1.2 (0.9 - 1.6)	3.3 (2.4 - 4.5)	1.7 (1.2 - 2.2)	0.208	**<0.001**	0.160
Glucose metabolism							
Fasting glucose, mmol/L	5.3 (5.2 - 5.4)	5.3 (5.2 - 5.4)	5.4 (5.3 - 5.5)	5.3 (5.2 - 5.4)	0.559	**<0.001**	0.069
Fasting insulin, pmol/L	48.3 (44.8 - 52.2)	44.0 (40.4 - 47.9)	51.1 (47.4 - 55)	47.3 (43.6 - 51.3)	0.220	**<0.001**	0.691
2-hr glucose, mmol/L	6.3 (5.9 - 6.7)	6.2 (5.8 - 6.5)	6.3 (6 - 6.7)	6.1 (5.8 - 6.4)	0.879	0.103	0.731
2-hr insulin, pmol/L	365.6 (322.1 - 415.9)	317.7 (278.6 - 361.4)	362.2 (319.9 - 410.2)	327.3 (289.1 - 369.8)	0.908	**0.002**	0.601
HOMA-IR, AU	1.7 (1.5 - 1.8)	1.5 (1.4 - 1.6)	1.8 (1.6 - 1.9)	1.6 (1.5 - 1.7)	0.228	**<0.001**	0.985
HOMA-β, AU	77.8 (72.3 - 83.8)	72.9 (67.5 - 78.9)	78.7 (73.3 - 84.5)	78.7 (73.1 - 84.7)	0.367	0.150	0.149
Matsuda index, AU	4.7 (4.2 - 5.1)	5.4 (4.9 - 5.9)	4.6 (4.2 - 5)	5.0 (4.5 - 5.4)	0.454	**<0.001**	0.213
Disposition index, AU	400 (359 - 445)	400 (360 - 445)	376 (339 - 417)	396 (358 - 439)	0.619	0.290	0.327
MISI, AU	0.129 (0.114 - 0.145)	0.153 (0.133 - 0.175)	0.114 (0.102 - 0.128)	0.130 (0.114 - 0.149)	0.059	**0.006**	0.722
HIRI, AU	397 (359 - 439)	324 (290 - 363)	391 (355 - 431)	361 (324 - 401)	0.511	**<0.001**	0.050
HbA1c, mmol/mol	36.2 (35.4 - 37.1)	35.8 (35.1 - 36.6)	36 (35.2 - 36.8)	35.8 (35.2 - 36.5)	0.836	0.093	0.501
Other parameters							
Fasting NEFA, mmol/L	0.46 (0.43 - 0.49)	0.44 (0.41 - 0.46)	0.47 (0.45 - 0.5)	0.44 (0.41 - 0.46)	0.681	**0.003**	0.361
Adipose tissue IR	22.7 (20.6 - 25.1)	19.7 (17.7 - 21.9)	24.0 (21.8 - 26.5)	21.3 (19.2 - 23.6)	0.312	**<0.001**	0.737
CRP, mg/L	1.1 (0.9 - 1.3)	0.9 (0.7 - 1.1)	1.1 (0.9 - 1.3)	1.0 (0.8 - 1.3)	0.663	**0.034**	0.101

[a] At MUMC+, VAT and liver fat were assessed using a whole-body MRI scan. [b] At WUR, VAT was assessed using single-slice MRI and liver fat was assessed using ^{1}H-MRS.
Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and centre. *P*-values <0.05 are highlighted in bold.
VAT, visceral adipose tissue; HOMA-IR, homeostatic model assessment of insulin resistance; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; FFA, free fatty acid; CRP, C-reactive protein.

Discussion

We previously demonstrated that precision nutrition based on tissue-specific IR phenotype enhanced improvements in cardiometabolic health, including a greater reduction in fasting serum TAG.[19] Here, we performed comprehensive plasma metabolite profiling to further characterise the effects of a 12-week LFHP or HMUFA diet on fasting and postprandial plasma metabolite profiles in individuals with predominant MIR or LIR. In individuals with MIR following the LFHP diet and individuals with LIR following the HMUFA diet, we observed greater reductions in fasting levels of plasma VLDL-TAG - more specifically TAG in the S-XL VLDL subclasses -, L VLDL particle concentrations, and VLDL particle size than in the other phenotype-diet combinations. We did not observe differential effects between the PhenoDiet groups on postprandial plasma metabolites; differential effects were more pronounced between the two diets than between the PhenoDiet groups. Irrespective of IR phenotype, the LFHP diet resulted in larger reductions in postprandial concentrations of almost all subclasses of VLDL, M LDL, and S HDL particles, as well as in in the TAG fractions of most subclasses of VLDL, LDL, and HDL subclasses, and BCAA and β-hydroxybutyrate concentrations, compared to the HMUFA diet. As for the fasting metabolite profile, the LFHP diet lowered plasma concentrations of L and XL VLDL and S HDL particles, as well as TAG fractions of almost all VLDL subclasses, S LDL, and S and M HDL, and β-hydroxybutyrate as compared to the HMUFA diet, all overlapping with the effects on postprandial metabolites.

Here, we show that the greater reduction in fasting TAG in individuals with MIR following the LFHP diet and individuals with LIR following the HMUFA diet we previously reported[19] was primarily due to a larger reduction of TAG in almost all VLDL subfractions, which carry most of the TAGs in plasma,[29] and not due to changes in TAG in the other lipoproteins. Fasting particle concentrations of L-VLDL and VLDL size were also decreased in these phenotype-diet combinations. Diet-induced effects on circulating lipids in combination with tissue-specific IR phenotypes have not been reported before. A reduction in fasting plasma VLDL TAG and large VLDL particles can result from decreased hepatic VLDL secretion, increased clearance from the circulation, or both. Hepatic VLDL production and secretion largely depend on substrate availability.[31,32] The previously reported diet-induced reductions in plasma NEFA, liver fat, and VAT[19] may have reduced substrate availability for VLDL production.[31,32] However, reductions in plasma NEFA, liver fat, and VAT were comparable between the PhenoDiet groups, hence not explaining the findings. As previously reported,[19] the LFHP diet lowered fasting plasma insulin levels in individuals with MIR, and the HMUFA diet reduced fasting plasma insulin in individuals with LIR, while fasting insulin was not decreased in the other phenotype-diet combinations. Insulin can stimulate VLDL production by promoting *de novo* lipogenesis (DNL) via activation of the transcription factor sterol regulatory element binding protein-1c (SREBP-1c),[33] and indeed, fasting insulin levels have been reported to positively correlate with fasting DNL.[34,35] As such, the reduction in fasting insulin levels may have contributed to the reduction in VLDL TAG and large VLDL particles in individuals with MIR following the LFHP diet and individuals with LIR following the HMUFA diet.

For postprandial metabolites, we observed differential effects between the two diets, but not between the PhenoDiet groups. Overall, the LFHP resulted in larger reductions in plasma concentrations of almost all subclasses of VLDL particles, S HDL particles, and TAG fractions in almost all VLDL subclasses and the smaller LDL and HDL subclasses, as compared to the HMUFA diet. Effects of a healthy diet enriched in protein or MUFA on several fasting blood lipids have been examined before in the OmniHeart Trial[36]; in this cross-over trial, 164 pre- or mildly hypertensive individuals followed three 6-week diets rich in fibre (~30 g/day) and low in SFA (6 en%), with substitution of 10 en% carbohydrates by either protein or MUFA. Although both the protein- and MUFA-enriched diets reduced fasting total cholesterol to a similar extent, the protein-enriched diet resulted in larger reductions in fasting plasma total TAG and VLDL-TAG compared to the MUFA-enriched diet, similar to our findings of larger reductions in VLDL-TAG upon the LFHP diet. Information on TAG in the different VLDL subclasses was not available in this trial. In the OmniHeart Trial, both diets were enriched in fibre, which points towards an independent lipid-lowering effect of increased protein consumption. This might suggest that the reduction in VLDL-TAG induced by the LFHP in our study, which was also enriched with fibre, might at least partly be due to the increased protein content of the diet. In line with this, a recent meta-analysis of 43 RCTs reported that higher-protein diets decreased fasting TAG compared to lower-protein diets[37] and 8-week supplementation of whey protein has also been shown to reduce plasma TAG.[38] The effects of increased consumption of dietary fibre or whole-grains on fasting plasma TAG are inconsistent, with some meta-analyses reporting TAG-lowering effects of whole-grain[39] or fibre intervention[40], and others reporting no effect.[41-44] Several studies have found high-fibre diets to reduce postprandial (VLDL-)TAG.[45-47] Lower-fat diets typically increase plasma TAG compared to higher-fat diets,[48,49] but these studies are unsuitable to directly compare to our study because the investigated low-fat diets are commonly higher in carbohydrates, which are known to increase blood lipids. In the present study, energy intake from carbohydrates was purposely kept similar among the two intervention diets.

Other trials have also reported minimal or no effects of high-MUFA diets on fasting plasma VLDL-TAG or VLDL particle concentrations.[50-52] With respect to total plasma TAG, a meta-analysis of 72 intervention studies concluded that increasing MUFA intake significantly lowers total serum TAG concentrations, but only to a minor

extent.[53] Importantly, however, these trials investigated the replacement of SFA by MUFA. In our study, both diets were low in SFA and enriched by either MUFA or protein and fibre; therefore, direct comparison is not possible. Interestingly, we observed a trend for a phenotype by diet interaction, with the LFHP diet reducing fasting VLDL-TAG and large VLDL particles in both individuals with MIR or LIR, while the HMUFA diet seemed to be only effective in individuals with LIR. Hence, our findings indicate that the (VLDL-)TAG-lowering potential of a MUFA-rich diet may depend on IR phenotype.

In summary, based on previous literature, the LFHP-induced larger reductions in fasting and postprandial VLDL particle concentrations and VLDL-TAG compared to the HMUFA diet may be partly due to the increased protein content of the diet. Effects of the fibre enrichment can also not be excluded, especially on reductions of postprandial VLDL-TAG.

A potential underlying mechanism for the LFHP-induced reduction in plasma VLDL may be the greater VAT reduction upon the LFHP diet, although this effect was only observed in a subgroup of the population in whom VAT was assessed using single-slice MRI. VAT is highly lipolytic,[54] and the LFHP-induced reduction in VAT may have decreased NEFA delivery to the liver, thereby reducing substrate availability for VLDL production and secretion. Reduced substrate availability is also supported by the observed reduction in fasting and postprandial concentrations of the ketone body β-hydroxybutyrate upon the LFHP diet, since ketogenesis is largely determined by NEFA delivery from adipose tissue lipolysis.[55] Both the observed reduction in VAT in a subpopulation and the reduction in plasma β-hydroxybutyrate point towards reduced substrate availability for VLDL production and secretion. Furthermore, the LFHP-induced improvement in hepatic insulin sensitivity may have reduced VLDL production by enhanced insulin-mediated suppression of VLDL production by promoting the hepatic degradation of ApoB.[56-59] The lower fat intake on the LFHP diet could have also lowered substrate availability: about 12-17% of TAG in fasting VLDL-TAG has been reported to be derived from dietary fat from previous meals via hepatic uptake of chylomicron remnants or chylomicron-derived NEFA that escaped uptake into peripheral tissues.[60,61] However, the day before the mixed-meal test, all participants consumed the same standardised meals irrespective of diet allocation. It is unclear how long this effect of lower fat intake would persist if present.

Furthermore, the high amount of fibre in the LFHP diet may have contributed to lower fasting and postprandial plasma VLDL-TAG via increased peripheral lipid uptake into adipose tissue, since fibre has been proposed to improve adipose tissue lipid storing capacity via the gut microbiota-mediated production of short-chain fatty acids (SCFA).[62,63] The mechanisms by which protein may reduce plasma VLDL-TAG remain speculative, but may be related to the high energy cost of the hepatic metabolic processing of protein, which is at least partly accountable for the increased dietary thermogenesis of high protein intake.[64,65] The mechanisms by which a diet low in fat and rich in protein and fibre can lower VLDL-TAG and VLDL particle concentrations requires further investigation.

As for HDL, the LFHP diet lowered fasting TAG in S and M HDL particles, and TAG in all HDL subclasses postprandially, while the HMUFA diet did not. A potential explanation may be decreased enzyme cholesteryl ester transfer protein (CETP) activity. CETP facilitates the transfer of TAG from TAG-rich VLDL particles to HDL and LDL in exchange for cholesteryl esters, resulting in the enrichment of HDL and LDL particles with TAG.[66] Higher serum CETP concentrations have indeed been associated with higher TAG content in S and M HDL particles.[67] Hence, the observed decreases in HDL-TAG may possibly result from decreased CETP activity. In addition, compared to the HMUFA diet, the LFHP diet reduced fasting and postprandial particle concentrations of S HDL particles without affecting particle concentrations of the larger HDL subclasses, which likely contributed to the observed increased average HDL size in the postprandial state upon this diet. These effects may also be explained by decreased CEPT activity since CETP promotes the formation of small HDL particles by TAG-enrichment of HDL; TAG-rich HDL is a preferred substrate for hepatic lipase, and hydrolysis of TAG from these HDL particles by hepatic lipase results in small HDL particles.[68] In line with this, higher serum CETP concentrations have been associated with smaller HDL size.[67] The increase in HDL size we observed after the LFHP diet may thus potentially be related to lower CETP action. Importantly, CETP activity is considered to be largely determined by plasma TAG, which is primarily contained in VLDL.[69,70] As VLDL and HDL metabolism are closely interlinked,[71] the LFHP-induced effects on circulating HDL particles may also result from the diet-induced reductions in VLDL-TAG.

Larger and less TAG-rich HDL particles have been proposed to be anti-atherogenic due to their higher capacity for reverse cholesterol transport and longer retention time in the circulation compared to smaller and TAG-rich HDL particles.[72] Indeed, larger HDL particle size has been associated with a reduced risk of future CVD.[73] Larger HDL size and lower TAG content in small HDL particles in fasting plasma have also been associated with a lower risk of T2DM.[74,75] Although little has been reported about the health effects of increased HDL size and reduced HDL-TAG in the postprandial state, these associations may be similar for postprandial blood lipids. Elevated fasting and postprandial plasma TAG - largely contained in VLDL particles - are well-established risk factors for cardiometabolic disease.[76-78] A reduction in plasma (VLDL-)TAG, as observed upon the LFHP diet, therefore confers important risk reduction. In addition, smaller VLDL size, observed upon the LFHP diet, has been associated with lower CVD and T2DM risk.[73,75] To summarise, compared to the HMUFA diet, the LFHP diet resulted in alterations in plasma VLDL and HDL profile that likely reduce cardiometabolic disease risk.

Furthermore, the LFHP diet lowered postprandial BCAA, while the HMUFA diet did not. Elevated fasting and postprandial BCAA are strongly associated to insulin resistance and T2DM, which is hypothesised to be largely attributable to dysregulated BCAA metabolism in adipose tissue, liver and skeletal muscle.[79,80] The LFHP diet also lowered postprandial plasma glutamine, an intermediary metabolite of BCAA metabolism that has also been found to be elevated in insulin resistance and obesity.[81] The few human trials that investigated a high-protein diet or supplementation with BCAA or whey protein on fasting plasma BCAA concentrations reported - in line with our findings – no effects on fasting BCAA concentrations, but did not assess postprandial BCAAs.[82-84] In animal studies, diets supplemented with casein or the BCAA leucine have been shown to increase the activity of enzymes involved in BCAA catabolism in liver and skeletal muscle, resulting in reduced plasma total BCAA concentrations.[85-88] The observed reduction in postprandial plasma BCAA by the LFHP diet appeared to be mainly accounted for by a steeper decrease after the peak one hour after the consumption of the mixed meal, suggesting faster BCAA degradation. It could be speculated that the increased daily intake of BCAA from protein in the LFHP diet upregulated BCAA catabolic pathways, thereby lowering postprandial plasma BCAA concentrations. Although increased BCAA intake via supplementation is more often implicated in exacerbating insulin resistance than vice versa in rodents, these detrimental effects of BCAA are mainly observed in combination with a high-fat diet.[81] Our findings suggest that in humans and in the context of a low-fat, high-fibre diet, moderately high BCAA intake from protein may benefit metabolic health.

As expected, the diets also had differential effects on plasma fatty acids, with the HMUFA diet increasing the plasma MUFA fraction and decreasing the PUFA fraction of fasting and postprandial total plasma FA, while the LFHP diet had opposite effects. Changes in plasma FA composition are largely determined by changes in dietary fat intake.[89] The observed changes most likely reflect the higher MUFA intake upon the HMUFA diet and the lower MUFA intake upon the LFHP diet.[19]

A strength of this study is the extensive plasma metabolic profiling in fasting conditions and at four time points in the postprandial state in a relatively large population of 214 women and men, which allowed for a detailed characterisation of the effects of LFHP and HMUFA diets on blood lipid profiles in two insulin resistance phenotypes. Furthermore, we purposely matched the two diets' carbohydrate and SFA content, which are well-known to affect plasma lipid profiles. Therefore, we could assess the effects of modification of the other dietary components without the interference of differences in carbohydrate or SFA content. The dietary intervention was implemented through intensive dietary counselling and the provision of key products, which resulted in high dietary compliance.

A limitation is that we sampled blood until four hours after consumption of the mixed meal and not for a more extended period. Many plasma lipids peak at four hours after a fat-rich meal, and therefore, for these lipids, we did not examine the (rate of) return to fasting levels. Future studies should employ longer sampling times of 6-8 hours to allow for a complete investigation of the postprandial plasma lipid response. We observed several interesting trends for phenotype-diet interactions, but this study was not powered to investigate effects in the four phenotype-diet combinations individually. Larger studies are needed to confirm the present findings. Importantly, this was an explorative study. Our results should be interpreted cautiously since many of the effects we observed were no longer statistically significant after adjustment for multiple testing. In addition, most of the differences in postprandial metabolites were found when comparing total metabolite exposure, i.e. total AUCs, and not when comparing metabolite iAUCs, which indicates that changes in fasting metabolite concentrations may have partly driven the diet effects we found on postprandial plasma metabolites.

Conclusion

In conclusion, the previously reported larger reductions in circulating fasting TAG in individuals with MIR following a LFHP diet and individuals with LIR following a HMUFA diet were due to a greater decrease of TAG in VLDL particles, and not in other lipoproteins. Our study suggests that a diet low in fat and rich in protein and fibre may be more effective than a diet rich in MUFA for improving plasma metabolite profile in individuals with tissue-specific IR, irrespective of IR phenotype. Compared to the HMUFA diet, the LFHP diet induced greater improvements in fasting and postprandial plasma VLDL and HDL profiles and larger reductions in plasma β-hydroxybutyrate and postprandial BCAA. As such, a LFHP diet may confer greater cardiometabolic risk reduction in individuals with either predominant liver or muscle insulin resistance compared to a HMUFA diet.

Acknowledgements

We would like to sincerely thank our study participants for their commitment. In addition, we thank W. Sluijsmans, H. Aydeniz, Y. Essers, and N. Hoebers for their support in performing lab analyses and E. Siebelink, H. Fick, K. Manusama, M. Grootte Bromhaar, O. Paling, M. Ummels, A. Hoge, S. Bowser, L. Omary, and all students for their invaluable contribution to the execution of the study.

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Supplementary material

These tables only include metabolite subclasses that were significantly affected by the intervention. Full supplemental tables can be found online: https://doi.org/10.4121/4c08e194-9715-4f5c-8515-55df53d2a3eb.

Table S1. Fasting plasma metabolites at week 0 and 12 in PhenoDiet groups A and B

	PhenoDiet A										PhenoDiet B										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Triglycerides																							
Total TAG, mmol/L	1.53	1.43	1.63	1.47	1.38	1.57	-0.14	-0.28	-0.01	**0.03**	1.69	1.57	1.82	1.57	1.45	1.70	-0.34	-0.47	-0.20	**<0.001**	**<0.001**	0.08	**0.04**
VLDL TAG, mmol/L	1.15	1.05	1.25	1.11	1.02	1.20	-0.12	-0.26	0.01	0.07	1.30	1.18	1.42	1.19	1.09	1.31	-0.32	-0.46	-0.19	**<0.001**	**<0.001**	0.08	**0.04**
LDL TAG, mmol/L	0.14	0.13	0.15	0.13	0.13	0.14	-0.23	-0.34	-0.13	**<0.001**	0.15	0.14	0.15	0.14	0.13	0.15	-0.33	-0.44	-0.22	**<0.001**	**<0.001**	0.27	0.24
HDL TAG, mmol/L	0.13	0.12	0.13	0.12	0.12	0.13	-0.13	-0.30	0.03	0.11	0.13	0.13	0.14	0.13	0.12	0.13	-0.26	-0.43	-0.10	**0.002**	**0.001**	0.44	0.27
Lipoprotein particle sizes																							
VLDL size, nm	39.6	39.3	39.8	39.7	39.4	39.9	-0.01	-0.14	0.12	0.91	39.9	39.6	40.2	39.7	39.5	40.0	-0.21	-0.34	-0.07	**0.002**	**0.02**	0.14	**0.04**
LDL size, nm	23.9	23.9	23.9	23.9	23.9	23.9	-0.05	-0.24	0.14	0.60	23.9	23.9	23.9	23.9	23.9	23.9	0.22	0.03	0.41	**0.03**	0.22	0.64	0.05
HDL size, nm	9.5	9.5	9.6	9.5	9.5	9.5	0.01	-0.08	0.10	0.81	9.5	9.5	9.6	9.5	9.5	9.6	0.08	-0.01	0.17	0.08	0.15	0.36	0.27
Other lipids																							
Phosphoglyc, mmol/L	2.34	2.27	2.41	2.18	2.11	2.25	-0.37	-0.48	-0.25	**<0.001**	2.35	2.28	2.42	2.22	2.15	2.29	-0.44	-0.56	-0.32	**<0.001**	**<0.001**	1.00	0.39
TAG/PG, ratio	0.65	0.61	0.70	0.68	0.63	0.72	0.02	-0.10	0.14	0.76	0.72	0.67	0.77	0.71	0.66	0.76	-0.17	-0.29	-0.04	**0.01**	0.10	**0.04**	**0.04**
Cholines, mmol/L	2.63	2.56	2.70	2.46	2.39	2.53	-0.38	-0.49	-0.27	**<0.001**	2.63	2.56	2.70	2.49	2.42	2.57	-0.45	-0.56	-0.33	**<0.001**	**<0.001**	0.91	0.41
Phosphatidylc, mmol/L	2.19	2.12	2.26	2.04	1.98	2.10	-0.35	-0.46	-0.23	**<0.001**	2.19	2.13	2.26	2.08	2.01	2.15	-0.44	-0.56	-0.32	**<0.001**	**<0.001**	0.98	0.29
Sphingomyelins, mmol/L	0.46	0.45	0.48	0.43	0.42	0.45	-0.40	-0.52	-0.29	**<0.001**	0.46	0.45	0.48	0.44	0.43	0.45	-0.46	-0.58	-0.35	**<0.001**	**<0.001**	0.79	0.47
Fatty acid ratios																							
Omega 3 %	3.2	3.0	3.4	3.2	3.0	3.4	-0.03	-0.17	0.11	0.65	3.3	3.0	3.5	3.3	3.0	3.6	-0.06	-0.20	0.08	0.40	0.36	0.95	0.78
Omega 6 %	37.9	37.5	38.4	38.3	37.8	38.7	0.13	-0.01	0.27	0.06	37.2	36.6	37.7	37.8	37.2	38.3	0.30	0.17	0.44	**<0.001**	**<0.001**	0.07	0.08
PUFA %	41.3	40.9	41.7	41.6	41.2	42.0	0.12	-0.02	0.26	0.09	40.6	40.0	41.2	41.3	40.7	41.8	0.31	0.17	0.45	**<0.001**	**<0.001**	0.09	0.06
MUFA %	25.5	25.1	25.9	26.0	25.6	26.4	0.16	0.04	0.29	**0.01**	26.0	25.5	26.5	26.1	25.6	26.6	-0.02	-0.15	0.11	0.79	0.11	0.27	**0.05**
SFA %	33.0	32.8	33.3	32.2	32.0	32.5	-0.48	-0.65	-0.32	**<0.001**	33.2	32.9	33.4	32.5	32.2	32.7	-0.53	-0.70	-0.37	**<0.001**	**<0.001**	0.26	0.68
LA %	29.9	29.5	30.4	30.0	29.6	30.5	0.04	-0.10	0.18	0.59	29.4	29.0	29.9	29.7	29.3	30.2	0.11	-0.03	0.26	0.12	0.14	0.18	0.46
DHA %	1.52	1.44	1.60	1.52	1.45	1.60	0.03	-0.13	0.18	0.75	1.49	1.41	1.57	1.51	1.40	1.62	<0.001	-0.16	0.16	0.96	0.80	0.45	0.85
PUFA/MUFA, ratio	1.62	1.58	1.66	1.60	1.56	1.64	-0.05	-0.18	0.08	0.47	1.56	1.51	1.61	1.58	1.53	1.63	0.15	0.02	0.28	**0.03**	0.29	0.17	**0.04**
Omega 6/Omega 3, ratio	11.8	11.0	12.6	12.0	11.2	12.8	0.05	-0.08	0.19	0.44	11.4	10.6	12.2	11.5	10.5	12.6	0.11	-0.03	0.25	0.11	0.09	0.68	0.55

Table S1. Continued

	PhenoDiet A										PhenoDiet B										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Lipoprotein subclasses																							
Very large VLDL																							
XL VLDL P, nmol/l	4.44	4.02	4.92	4.30	3.90	4.74	-0.12	-0.25	0.02	0.09	5.10	4.56	5.69	4.66	4.16	5.22	-0.30	-0.44	-0.17	**<0.001**	**<0.001**	0.10	0.05
XL VLDL L, mmol/L	0.261	0.235	0.289	0.251	0.228	0.278	-0.12	-0.25	0.01	0.08	0.301	0.269	0.336	0.274	0.244	0.306	-0.31	-0.44	-0.17	**<0.001**	**<0.001**	0.08	0.05
XL VLDL PL, mmol/L	0.048	0.044	0.054	0.047	0.042	0.051	-0.12	-0.25	0.01	0.08	0.056	0.050	0.063	0.051	0.045	0.057	-0.28	-0.42	-0.15	**<0.001**	**<0.001**	0.10	0.08
XL VLDL C, mmol/L	0.066	0.061	0.072	0.063	0.058	0.068	-0.17	-0.28	-0.05	**0.01**	0.074	0.067	0.081	0.068	0.061	0.075	-0.31	-0.43	-0.19	**<0.001**	**<0.001**	0.13	0.09
XL VLDL CE, mmol/L	0.036	0.034	0.039	0.034	0.031	0.037	-0.20	-0.31	-0.09	**<0.001**	0.040	0.036	0.044	0.036	0.033	0.040	-0.33	-0.44	-0.22	**<0.001**	**<0.001**	0.19	0.10
XL VLDL FC, mmol/L	0.030	0.027	0.032	0.028	0.026	0.031	-0.13	-0.26	<0.001	0.05	0.034	0.030	0.037	0.031	0.028	0.035	-0.29	-0.42	-0.15	**<0.001**	**<0.001**	0.09	0.10
XL VLDL TAG, mmol/L	0.145	0.129	0.162	0.141	0.126	0.158	-0.10	-0.24	0.04	0.18	0.170	0.151	0.191	0.154	0.136	0.173	-0.31	-0.45	-0.16	**<0.001**	**<0.001**	0.07	**0.04**
Large VLDL																							
L VLDL P, nmol/l	13.8	12.6	15.1	13.4	12.3	14.7	-0.12	-0.25	0.01	0.07	15.6	14.2	17.2	14.3	12.9	15.9	-0.32	-0.45	-0.19	**<0.001**	**<0.001**	0.11	**0.03**
L VLDL L, mmol/L	0.459	0.420	0.503	0.443	0.405	0.485	-0.13	-0.26	<0.001	**0.05**	0.518	0.470	0.570	0.474	0.428	0.526	-0.33	-0.46	-0.20	**<0.001**	**<0.001**	0.11	**0.04**
L VLDL PL, mmol/L	0.088	0.080	0.097	0.086	0.078	0.094	-0.11	-0.24	0.02	0.09	0.100	0.090	0.111	0.092	0.082	0.103	-0.30	-0.43	-0.17	**<0.001**	**<0.001**	0.13	**0.04**
L VLDL C, mmol/L	0.127	0.117	0.138	0.122	0.113	0.132	-0.14	-0.26	-0.02	**0.02**	0.140	0.128	0.154	0.131	0.119	0.144	-0.30	-0.42	-0.18	**<0.001**	**<0.001**	0.16	0.06
L VLDL CE, mmol/L	0.065	0.061	0.070	0.063	0.058	0.068	-0.15	-0.26	-0.04	**0.01**	0.071	0.065	0.078	0.067	0.061	0.074	-0.29	-0.40	-0.17	**<0.001**	**<0.001**	0.20	0.10
L VLDL FC, mmol/L	0.062	0.056	0.067	0.059	0.055	0.065	-0.13	-0.26	-0.01	**0.04**	0.069	0.062	0.076	0.064	0.057	0.070	-0.31	-0.44	-0.19	**<0.001**	**<0.001**	0.13	**0.05**
L VLDL TAG, mmol/L	0.243	0.220	0.267	0.234	0.212	0.258	-0.13	-0.26	0.01	0.06	0.276	0.250	0.305	0.250	0.225	0.278	-0.34	-0.48	-0.20	**<0.001**	**<0.001**	0.09	**0.03**
Medium VLDL																							
M VLDL P, nmol/l	44.1	41.6	46.8	40.9	38.4	43.5	-0.26	-0.36	-0.15	**<0.001**	46.8	43.7	50.2	43.1	39.9	46.6	-0.38	-0.49	-0.27	**<0.001**	**<0.001**	0.24	0.10
M VLDL L, mmol/L	0.747	0.703	0.794	0.699	0.656	0.745	-0.23	-0.35	-0.12	**<0.001**	0.800	0.747	0.858	0.736	0.680	0.796	-0.39	-0.51	-0.28	**<0.001**	**<0.001**	0.20	0.05
M VLDL PL, mmol/L	0.163	0.153	0.173	0.149	0.140	0.159	-0.27	-0.38	-0.17	**<0.001**	0.172	0.160	0.185	0.157	0.145	0.171	-0.38	-0.49	-0.28	**<0.001**	**<0.001**	0.28	0.14
M VLDL C, mmol/L	0.208	0.196	0.220	0.185	0.173	0.198	-0.33	-0.43	-0.23	**<0.001**	0.213	0.199	0.228	0.196	0.180	0.213	-0.35	-0.45	-0.25	**<0.001**	**<0.001**	0.53	0.80
M VLDL CE, mmol/L	0.108	0.101	0.115	0.094	0.087	0.102	-0.33	-0.43	-0.22	**<0.001**	0.107	0.100	0.116	0.100	0.091	0.109	-0.28	-0.38	-0.17	**<0.001**	**<0.001**	0.87	0.52
M VLDL FC, mmol/L	0.099	0.093	0.105	0.090	0.084	0.096	-0.30	-0.40	-0.19	**<0.001**	0.104	0.097	0.112	0.095	0.088	0.104	-0.38	-0.49	-0.28	**<0.001**	**<0.001**	0.30	0.23
M VLDL TAG, mmol/L	0.370	0.343	0.399	0.358	0.331	0.386	-0.14	-0.27	-0.01	**0.04**	0.408	0.376	0.442	0.375	0.344	0.410	-0.35	-0.49	-0.22	**<0.001**	**<0.001**	0.15	**0.02**
Small VLDL																							
S VLDL P, nmol/l	44.7	42.4	47.0	42.4	40.3	44.5	-0.22	-0.33	-0.10	**<0.001**	47.4	44.8	50.2	45.1	42.3	48.1	-0.34	-0.45	-0.22	**<0.001**	**<0.001**	0.17	0.15
S VLDL L, mmol/L	0.463	0.440	0.486	0.436	0.415	0.457	-0.24	-0.35	-0.13	**<0.001**	0.489	0.462	0.517	0.463	0.435	0.492	-0.35	-0.47	-0.24	**<0.001**	**<0.001**	0.18	0.17
S VLDL PL, mmol/L	0.112	0.106	0.117	0.103	0.098	0.109	-0.30	-0.40	-0.19	**<0.001**	0.116	0.110	0.123	0.109	0.103	0.117	-0.36	-0.47	-0.26	**<0.001**	**<0.001**	0.29	0.39
S VLDL C, mmol/L	0.171	0.163	0.180	0.156	0.147	0.165	-0.32	-0.43	-0.22	**<0.001**	0.177	0.167	0.187	0.166	0.155	0.177	-0.34	-0.44	-0.23	**<0.001**	**<0.001**	0.37	0.85
S VLDL CE, mmol/L	0.103	0.098	0.109	0.094	0.089	0.100	-0.31	-0.41	-0.20	**<0.001**	0.106	0.100	0.113	0.100	0.094	0.107	-0.31	-0.42	-0.20	**<0.001**	**<0.001**	0.37	0.98
S VLDL FC, mmol/L	0.068	0.065	0.072	0.062	0.058	0.065	-0.34	-0.44	-0.24	**<0.001**	0.070	0.066	0.074	0.065	0.061	0.070	-0.37	-0.48	-0.27	**<0.001**	**<0.001**	0.38	0.65
S VLDL TAG, mmol/L	0.177	0.167	0.188	0.174	0.164	0.184	-0.09	-0.23	0.04	0.18	0.193	0.181	0.205	0.184	0.172	0.198	-0.30	-0.44	-0.16	**<0.001**	**<0.001**	0.12	**0.04**

Table S1. Continued

	PhenoDiet A										PhenoDiet B										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Very large HDL																							
XL HDL P, nmol/l	201.3	190.7	212.4	187.9	178.5	197.8	-0.14	-0.24	-0.05	**0.004**	198.6	186.8	211.1	192.4	182.2	203.1	-0.08	-0.18	0.02	0.11	**0.002**	0.91	0.38
XL HDL L, mmol/L	0.136	0.127	0.146	0.129	0.121	0.138	-0.05	-0.14	0.05	0.36	0.130	0.120	0.142	0.131	0.123	0.141	0.06	-0.04	0.16	0.23	0.84	0.64	0.13
XL HDL PL, mmol/L	0.058	0.053	0.063	0.055	0.050	0.060	0.002	-0.08	0.08	0.97	0.051	0.044	0.060	0.056	0.051	0.062	0.12	0.04	0.19	**0.004**	**0.04**	0.44	**0.04**
XL HDL C, mmol/L	0.071	0.067	0.075	0.067	0.064	0.071	-0.08	-0.18	0.02	0.10	0.069	0.064	0.074	0.068	0.064	0.072	0.001	-0.10	0.10	0.99	0.25	0.57	0.24
XL HDL CE, mmol/L	0.049	0.046	0.053	0.046	0.043	0.049	-0.08	-0.17	0.01	0.10	0.047	0.043	0.051	0.046	0.043	0.050	0.01	-0.08	0.10	0.84	0.31	0.51	0.19
XL HDL FC, mmol/L	0.022	0.021	0.022	0.021	0.020	0.022	-0.06	-0.18	0.05	0.29	0.021	0.020	0.022	0.021	0.020	0.022	0.005	-0.12	0.13	0.94	0.48	0.93	0.42
XL HDL TAG, mmol/L	0.006	0.006	0.006	0.006	0.005	0.006	-0.19	-0.33	-0.06	**0.01**	0.006	0.006	0.007	0.006	0.006	0.007	-0.30	-0.44	-0.16	**<0.001**	**<0.001**	0.12	0.27
Relative lipoprotein lipid concentrations																							
Very large VLDL																							
XL VLDL PL %	18.6	18.3	18.8	18.5	18.3	18.7	-0.03	-0.20	0.14	0.72	18.5	18.3	18.8	18.7	18.4	18.9	0.15	-0.02	0.33	0.08	0.31	0.96	0.13
XL VLDL C %	25.4	24.6	26.1	24.9	24.2	25.7	-0.08	-0.25	0.08	0.31	24.5	23.8	25.2	24.8	24.0	25.5	0.17	0.003	0.33	**0.05**	0.47	0.13	**0.03**
XL VLDL CE %	14.0	13.4	14.6	13.5	13.0	14.1	-0.10	-0.27	0.07	0.24	13.2	12.7	13.8	13.3	12.7	14.0	0.12	-0.05	0.29	0.16	0.86	0.10	0.07
XL VLDL FC %	11.3	11.1	11.5	11.3	11.1	11.5	-0.01	-0.18	0.17	0.94	11.2	11.0	11.4	11.4	11.2	11.6	0.27	0.09	0.45	**0.003**	**0.04**	0.39	**0.03**
XL VLDL TAG %	55.5	54.6	56.5	56.1	55.0	57.1	0.08	-0.09	0.25	0.33	56.5	55.7	57.3	56.2	55.3	57.1	-0.17	-0.34	0.005	0.06	0.50	0.18	**0.04**
Large VLDL																							
L VLDL PL %	19.2	18.9	19.4	19.3	19.1	19.5	0.10	-0.03	0.23	0.13	19.2	19.0	19.5	19.4	19.1	19.6	0.05	-0.08	0.19	0.42	0.10	0.85	0.62
L VLDL C %	27.7	27.1	28.3	27.6	27.0	28.2	0.01	-0.16	0.17	0.95	27.1	26.6	27.7	27.6	27.0	28.2	0.24	0.07	0.40	**0.005**	**0.04**	0.18	**0.05**
L VLDL CE %	14.2	13.8	14.7	14.1	13.7	14.6	0.004	-0.15	0.16	0.96	13.8	13.3	14.2	14.1	13.6	14.6	0.24	0.08	0.39	**0.003**	**0.03**	0.16	**0.04**
L VLDL FC %	13.4	13.2	13.5	13.4	13.3	13.6	0.02	-0.15	0.19	0.81	13.3	13.2	13.4	13.4	13.2	13.6	0.20	0.03	0.37	**0.02**	0.07	0.37	0.14
L VLDL TAG %	52.9	52.1	53.6	52.8	52.1	53.6	-0.03	-0.19	0.13	0.71	53.4	52.8	54.0	52.8	52.0	53.5	-0.22	-0.39	-0.06	**0.01**	**0.03**	0.29	0.10
Medium VLDL																							
M VLDL PL %	21.8	21.5	22.0	21.3	21.0	21.6	-0.21	-0.35	-0.07	**0.004**	21.5	21.2	21.8	21.4	21.0	21.8	0.01	-0.13	0.15	0.88	0.06	0.37	**0.03**
M VLDL C %	27.8	26.8	28.9	26.5	25.3	27.7	-0.17	-0.31	-0.03	**0.02**	26.6	25.6	27.7	26.6	25.5	27.8	0.07	-0.07	0.21	0.33	0.34	0.25	**0.02**
M VLDL CE %	14.5	13.7	15.3	13.5	12.6	14.4	-0.14	-0.28	-0.01	**0.04**	13.4	12.6	14.3	13.6	12.7	14.4	0.09	-0.05	0.23	0.20	0.58	0.22	**0.02**
M VLDL FC %	13.3	13.0	13.5	12.9	12.6	13.2	-0.20	-0.35	-0.06	**0.01**	13.0	12.8	13.3	13.0	12.7	13.3	0.01	-0.13	0.16	0.85	0.07	0.44	**0.04**
M VLDL TAG %	49.5	48.2	50.8	51.2	49.8	52.6	0.18	0.03	0.34	**0.02**	50.9	49.7	52.2	51.0	49.6	52.5	-0.07	-0.23	0.09	0.39	0.31	0.27	**0.03**
Small VLDL																							
S VLDL PL %	24.2	23.9	24.4	23.7	23.4	24.0	-0.26	-0.40	-0.12	**<0.001**	23.8	23.5	24.1	23.6	23.3	24.0	-0.05	-0.19	0.09	0.45	**0.002**	0.25	**0.04**
S VLDL C %	37.0	36.3	37.8	35.8	34.9	36.7	-0.23	-0.38	-0.09	**0.002**	36.2	35.4	37.0	35.9	35.0	36.8	-0.01	-0.15	0.14	0.94	**0.02**	0.32	**0.03**
S VLDL CE %	22.3	21.8	22.8	21.7	21.1	22.3	-0.18	-0.33	-0.04	**0.02**	21.8	21.3	22.3	21.7	21.1	22.3	0.05	-0.10	0.20	0.51	0.21	0.37	**0.03**
S VLDL FC %	14.7	14.4	15.0	14.1	13.8	14.5	-0.30	-0.44	-0.17	**<0.001**	14.4	14.1	14.7	14.1	13.8	14.5	-0.11	-0.24	0.03	0.13	**<0.001**	0.28	**0.05**
S VLDL TAG %	38.3	37.3	39.3	39.9	38.8	41.0	0.25	0.09	0.41	**0.002**	39.4	38.4	40.5	39.9	38.7	41.1	0.01	-0.15	0.17	0.94	**0.02**	0.34	**0.03**

Table S1. Continued

	PhenoDiet A										PhenoDiet B										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Very large HDL																							
XL HDL PL %	42.4	41.2	43.5	42.6	41.3	44.0	0.04	-0.02	0.11	0.19	39.4	35.9	43.3	42.9	41.8	44.1	0.15	0.09	0.22	**<0.001**	**0.002**	0.35	**0.02**
XL HDL C %	52.4	51.6	53.1	52.2	51.4	53.1	-0.12	-0.24	<0.001	**0.05**	52.7	51.9	53.6	51.6	50.8	52.4	-0.30	-0.42	-0.18	**<0.001**	**<0.001**	0.95	**0.03**
XL HDL CE %	36.2	35.8	36.7	35.8	35.3	36.3	-0.13	-0.25	-0.01	**0.03**	36.0	35.4	36.6	35.4	34.9	35.9	-0.21	-0.33	-0.09	**<0.001**	**<0.001**	0.25	0.40
XL HDL FC %	15.9	15.3	16.5	16.3	15.6	16.9	0.02	-0.08	0.11	0.72	16.4	15.7	17.1	16.1	15.5	16.7	-0.11	-0.21	-0.02	**0.02**	0.15	0.40	0.05
XL HDL TAG %	4.4	4.1	4.8	4.3	4.1	4.7	-0.08	-0.20	0.04	0.20	4.9	4.5	5.4	4.7	4.3	5.1	-0.24	-0.37	-0.12	**<0.001**	**<0.001**	0.17	0.07

Values are geometric means with 95% confidence intervals. The differences in fasting plasma metabolites between PhenoDiet groups A and B were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates.

Table S2. Fasting plasma metabolites at week 0 and 12 upon LFHP or HMUFA diet

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Triglycerides																							
Total TAG, mmol/L	1.72	1.60	1.84	1.51	1.41	1.62	-0.35	-0.48	-0.21	**<0.001**	1.59	1.48	1.72	1.52	1.42	1.64	-0.14	-0.27	-0.01	**0.031**	**<0.001**	0.406	**0.036**
VLDL TAG, mmol/L	1.32	1.21	1.43	1.14	1.05	1.24	-0.33	-0.46	-0.19	**<0.001**	1.20	1.10	1.31	1.15	1.05	1.26	-0.13	-0.26	0.01	0.061	**<0.001**	0.361	**0.039**
LDL TAG, mmol/L	0.15	0.14	0.15	0.14	0.13	0.14	-0.33	-0.44	-0.22	**<0.001**	0.14	0.14	0.15	0.14	0.13	0.14	-0.23	-0.34	-0.12	**<0.001**	**<0.001**	0.754	0.185
HDL TAG, mmol/L	0.13	0.13	0.14	0.12	0.12	0.13	-0.34	-0.51	-0.18	**<0.001**	0.13	0.12	0.14	0.13	0.12	0.13	-0.06	-0.22	0.10	0.436	**0.001**	0.893	**0.018**
Lipoprotein particle sizes																							
VLDL size, nm	39.9	39.7	40.1	39.6	39.4	39.9	-0.21	-0.35	-0.08	**0.002**	39.7	39.4	39.9	39.7	39.4	40.0	-0.01	-0.13	0.12	0.934	**0.020**	0.487	**0.027**
LDL size, nm	23.9	23.9	23.9	23.9	23.9	23.9	0.23	0.03	0.42	**0.023**	23.9	23.9	23.9	23.9	23.9	23.9	-0.05	-0.24	0.14	0.599	0.198	0.884	**0.044**
HDL size, nm	9.5	9.5	9.5	9.5	9.5	9.5	0.09	0.00	0.18	0.059	9.5	9.5	9.6	9.5	9.5	9.6	0.01	-0.08	0.10	0.884	0.142	0.158	0.205
Fatty acids																							
Total FA, mmol/L	13.4	13.0	13.9	12.4	12.0	12.9	-0.43	-0.55	-0.31	**<0.001**	13.2	12.8	13.7	12.5	12.1	13.0	-0.31	-0.42	-0.19	**<0.001**	**<0.001**	0.824	0.136
Omega 3, mmol/L	0.45	0.41	0.49	0.44	0.40	0.48	-0.08	-0.20	0.05	0.218	0.43	0.40	0.46	0.38	0.35	0.42	-0.31	-0.42	-0.19	**<0.001**	**<0.001**	0.127	**0.010**
Omega 6, mmol/L	5.0	4.9	5.1	4.7	4.6	4.9	-0.40	-0.53	-0.28	**<0.001**	5.0	4.8	5.1	4.7	4.6	4.9	-0.34	-0.46	-0.22	**<0.001**	**<0.001**	0.979	0.468
PUFA, mmol/L	5.5	5.3	5.6	5.2	5.0	5.4	-0.37	-0.49	-0.25	**<0.001**	5.4	5.3	5.6	5.1	5.0	5.3	-0.35	-0.46	-0.24	**<0.001**	**<0.001**	0.731	0.835
MUFA, mmol/L	3.5	3.3	3.7	3.2	3.0	3.3	-0.39	-0.51	-0.27	**<0.001**	3.4	3.2	3.6	3.3	3.1	3.5	-0.13	-0.24	-0.02	**0.026**	**<0.001**	0.937	**0.002**
SFA, mmol/L	4.5	4.3	4.6	4.0	3.9	4.2	-0.49	-0.62	-0.36	**<0.001**	4.4	4.2	4.6	4.1	3.9	4.2	-0.40	-0.52	-0.27	**<0.001**	**<0.001**	0.839	0.306
LA, mmol/L	4.0	3.8	4.1	3.7	3.6	3.8	-0.40	-0.53	-0.28	**<0.001**	3.9	3.8	4.1	3.7	3.6	3.9	-0.29	-0.41	-0.17	**<0.001**	**<0.001**	0.921	0.209
DHA, mmol/L	0.20	0.19	0.22	0.20	0.19	0.21	-0.11	-0.24	0.03	0.120	0.20	0.19	0.21	0.18	0.17	0.20	-0.30	-0.43	-0.18	**<0.001**	**<0.001**	0.226	**0.035**

Table S2. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Fatty acid ratios																							
Omega 3 %	3.3	3.1	3.5	3.6	3.3	3.8	0.14	0.01	0.28	**0.041**	3.3	3.1	3.4	3.0	2.8	3.3	-0.22	-0.35	-0.08	**0.001**	0.453	0.068	**<0.001**
Omega 6 %	37.1	36.5	37.6	38.1	37.5	38.6	0.33	0.19	0.47	**<0.001**	37.6	37.1	38.0	37.8	37.4	38.3	0.11	-0.02	0.24	0.104	**<0.001**	0.533	**0.024**
PUFA %	40.6	40.1	41.1	41.8	41.3	42.3	0.40	0.27	0.54	**<0.001**	41.0	40.5	41.5	41.1	40.6	41.5	0.05	-0.09	0.18	0.506	**<0.001**	0.823	**<0.001**
MUFA %	26.1	25.7	26.5	25.6	25.2	26.0	-0.18	-0.30	-0.06	**0.004**	25.6	25.2	26.0	26.4	25.9	26.8	0.31	0.19	0.42	**<0.001**	0.143	0.797	**<0.001**
SFA %	33.1	32.9	33.4	32.4	32.2	32.7	-0.44	-0.61	-0.28	**<0.001**	33.2	33.0	33.5	32.4	32.2	32.7	-0.57	-0.73	-0.40	**<0.001**	**<0.001**	0.971	0.308
LA %	29.4	28.9	29.9	29.8	29.3	30.3	0.10	-0.04	0.25	0.169	29.7	29.3	30.1	29.8	29.4	30.2	0.05	-0.09	0.19	0.457	0.132	0.439	0.630
DHA %	1.5	1.4	1.6	1.6	1.5	1.7	0.19	0.03	0.35	**0.020**	1.5	1.4	1.6	1.5	1.4	1.5	-0.14	-0.29	0.01	0.067	0.673	0.198	**0.003**
PUFA/MUFA, ratio	1.6	1.5	1.6	1.6	1.6	1.7	0.28	0.16	0.41	**<0.001**	1.6	1.6	1.6	1.6	1.5	1.6	-0.17	-0.29	-0.05	**0.007**	0.181	0.804	**<0.001**
Omega 6/Omega 3, ratio	11.2	10.4	12.0	10.7	9.9	11.5	-0.07	-0.21	0.07	0.302	11.5	10.9	12.3	12.5	11.6	13.5	0.22	0.09	0.35	**0.001**	0.121	0.068	**0.003**
Glycolysis-related metabolites																							
Lactate, mmol/L	1.05	0.99	1.11	1.06	0.99	1.12	0.05	-0.16	0.26	0.636	1.03	0.98	1.09	1.06	1.01	1.13	0.10	-0.10	0.30	0.309	0.296	0.895	0.717
Pyruvate, mmol/L	0.06	0.05	0.06	0.06	0.05	0.06	0.19	-0.02	0.40	0.074	0.06	0.05	0.06	0.07	0.06	0.07	0.42	0.23	0.62	**<0.001**	**<0.001**	**0.022**	0.112
Citrate, mmol/L	0.08	0.08	0.08	0.08	0.08	0.08	-0.09	-0.30	0.11	0.375	0.08	0.08	0.08	0.08	0.08	0.09	0.26	0.07	0.46	**0.009**	0.241	**0.034**	**0.015**
Ketone bodies																							
3-Hydroxybutyrate, mmol/L	0.042	0.037	0.049	0.031	0.027	0.036	-0.44	-0.65	-0.24	**<0.001**	0.047	0.040	0.055	0.043	0.038	0.049	-0.13	-0.33	0.07	0.187	**<0.001**	**0.009**	**0.034**
Acetate, mmol/L	0.032	0.030	0.034	0.034	0.030	0.039	0.14	-0.11	0.38	0.272	0.034	0.032	0.036	0.035	0.032	0.038	0.05	-0.19	0.28	0.690	0.285	0.390	0.602
Acetoacetate, mmol/L	0.023	0.020	0.025	0.020	0.018	0.023	-0.20	-0.42	0.01	0.059	0.025	0.022	0.028	0.025	0.022	0.028	0.00	-0.21	0.20	0.979	0.167	**0.016**	0.178
Acetone, mmol/L	0.015	0.014	0.015	0.014	0.014	0.015	-0.10	-0.31	0.11	0.329	0.015	0.014	0.016	0.015	0.015	0.016	0.05	-0.15	0.25	0.608	0.727	**0.048**	0.290
Lipoprotein subclasses																							
Very large VLDL																							
XL VLDL P, nmol/L	5.26	4.76	5.80	4.47	4.04	4.94	-0.31	-0.45	-0.17	**<0.001**	4.65	4.17	5.18	4.45	4.00	4.94	-0.12	-0.25	0.02	0.083	**<0.001**	0.264	**0.045**
XL VLDL L, mmol/L	0.309	0.280	0.341	0.262	0.236	0.290	-0.31	-0.45	-0.17	**<0.001**	0.309	0.280	0.341	0.262	0.236	0.290	-0.12	-0.25	0.01	0.068	**<0.001**	0.279	0.051
XL VLDL PL, mmol/L	0.057	0.052	0.063	0.049	0.044	0.054	-0.29	-0.42	-0.15	**<0.001**	0.057	0.052	0.063	0.049	0.044	0.054	-0.12	-0.25	0.01	0.063	**<0.001**	0.251	0.089
XL VLDL C, mmol/L	0.075	0.069	0.082	0.066	0.060	0.072	-0.31	-0.43	-0.19	**<0.001**	0.075	0.069	0.082	0.066	0.060	0.072	-0.17	-0.28	-0.05	**0.004**	**<0.001**	0.319	0.085
XL VLDL CE, mmol/L	0.041	0.037	0.044	0.035	0.032	0.039	-0.33	-0.45	-0.22	**<0.001**	0.041	0.037	0.044	0.035	0.032	0.039	-0.20	-0.30	-0.09	**<0.001**	**<0.001**	0.373	0.080
XL VLDL FC, mmol/L	0.034	0.031	0.038	0.030	0.027	0.033	-0.29	-0.42	-0.15	**<0.001**	0.034	0.031	0.038	0.030	0.027	0.033	-0.13	-0.26	0.00	**0.045**	**<0.001**	0.268	0.097
XL VLDL TAG, mmol/L	0.175	0.157	0.194	0.146	0.130	0.163	-0.31	-0.46	-0.17	**<0.001**	0.175	0.157	0.194	0.146	0.130	0.163	-0.10	-0.24	0.04	0.166	**<0.001**	0.290	**0.037**
Large VLDL																							
L VLDL P, nmol/L	16.0	14.7	17.5	13.8	12.6	15.1	-0.32	-0.46	-0.19	**<0.001**	14.4	13.1	15.9	13.8	12.6	15.1	-0.12	-0.25	0.01	0.062	**<0.001**	0.307	**0.031**
L VLDL L, mmol/L	0.530	0.486	0.579	0.455	0.415	0.499	-0.33	-0.47	-0.20	**<0.001**	0.530	0.486	0.579	0.455	0.415	0.499	-0.13	-0.26	-0.01	**0.040**	**<0.001**	0.336	**0.036**
L VLDL PL, mmol/L	0.103	0.094	0.113	0.088	0.080	0.097	-0.31	-0.44	-0.18	**<0.001**	0.103	0.094	0.113	0.088	0.080	0.097	-0.11	-0.24	0.02	0.087	**<0.001**	0.283	**0.032**

Table S2. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Large VLDL																							
L VLDL C, mmol/L	0.144	0.133	0.156	0.127	0.116	0.139	-0.31	-0.43	-0.18	**<0.001**	0.144	0.133	0.156	0.127	0.116	0.139	-0.14	-0.26	-0.03	**0.016**	**<0.001**	0.321	0.058
L VLDL CE, mmol/L	0.073	0.067	0.079	0.065	0.060	0.071	-0.29	-0.41	-0.17	**<0.001**	0.073	0.067	0.079	0.065	0.060	0.071	-0.15	-0.26	-0.04	**0.009**	**<0.001**	0.356	0.092
L VLDL FC, mmol/L	0.071	0.065	0.077	0.061	0.056	0.067	-0.32	-0.44	-0.19	**<0.001**	0.071	0.065	0.077	0.061	0.056	0.067	-0.13	-0.25	-0.01	**0.034**	**<0.001**	0.294	**0.039**
L VLDL TAG, mmol/L	0.282	0.257	0.309	0.238	0.216	0.263	-0.34	-0.48	-0.20	**<0.001**	0.282	0.257	0.309	0.238	0.216	0.263	-0.13	-0.27	0.00	0.051	**<0.001**	0.389	**0.033**
Medium VLDL																							
M VLDL P, nmol/L	47.6	44.7	50.7	42.4	39.4	45.6	-0.37	-0.48	-0.26	**<0.001**	45.2	42.4	48.1	42.0	39.4	45.6	-0.27	-0.37	-0.17	**<0.001**	**<0.001**	0.404	0.183
M VLDL L, mmol/L	0.814	0.763	0.869	0.719	0.669	0.773	-0.39	-0.50	-0.27	**<0.001**	0.814	0.763	0.869	0.719	0.669	0.773	-0.24	-0.35	-0.13	**<0.001**	**<0.001**	0.437	0.080
M VLDL PL, mmol/L	0.175	0.164	0.187	0.155	0.143	0.167	-0.37	-0.48	-0.27	**<0.001**	0.175	0.164	0.187	0.155	0.143	0.167	-0.29	-0.39	-0.18	**<0.001**	**<0.001**	0.472	0.233
M VLDL C, mmol/L	0.216	0.203	0.230	0.195	0.179	0.212	-0.34	-0.44	-0.24	**<0.001**	0.216	0.203	0.230	0.195	0.179	0.212	-0.34	-0.44	-0.24	**<0.001**	**<0.001**	0.565	0.984
M VLDL CE, mmol/L	0.109	0.102	0.117	0.100	0.091	0.109	-0.28	-0.39	-0.17	**<0.001**	0.109	0.102	0.117	0.100	0.091	0.109	-0.32	-0.42	-0.22	**<0.001**	**<0.001**	0.685	0.570
M VLDL FC, mmol/L	0.106	0.099	0.113	0.094	0.087	0.102	-0.37	-0.47	-0.26	**<0.001**	0.106	0.099	0.113	0.094	0.087	0.102	-0.31	-0.41	-0.21	**<0.001**	**<0.001**	0.472	0.467
M VLDL TAG, mmol/L	0.416	0.386	0.449	0.362	0.334	0.392	-0.36	-0.50	-0.23	**<0.001**	0.416	0.386	0.449	0.362	0.334	0.392	-0.14	-0.26	-0.01	**0.039**	**<0.001**	0.454	**0.018**
Small VLDL																							
S VLDL P, nmol/L	48.1	45.6	50.6	44.0	41.6	46.5	-0.35	-0.46	-0.23	**<0.001**	45.9	43.4	48.5	43.8	41.4	46.5	-0.21	-0.32	-0.10	**<0.001**	**<0.001**	0.459	0.092
S VLDL L, mmol/L	0.495	0.471	0.521	0.452	0.428	0.478	-0.36	-0.48	-0.25	**<0.001**	0.495	0.471	0.521	0.452	0.428	0.478	-0.23	-0.35	-0.12	**<0.001**	**<0.001**	0.467	0.111
S VLDL PL, mmol/L	0.118	0.112	0.124	0.108	0.101	0.114	-0.36	-0.47	-0.26	**<0.001**	0.118	0.112	0.124	0.108	0.101	0.114	-0.30	-0.40	-0.19	**<0.001**	**<0.001**	0.480	0.376
S VLDL C, mmol/L	0.179	0.170	0.189	0.164	0.153	0.175	-0.35	-0.46	-0.24	**<0.001**	0.179	0.170	0.189	0.164	0.153	0.175	-0.31	-0.41	-0.21	**<0.001**	**<0.001**	0.471	0.611
S VLDL CE, mmol/L	0.108	0.103	0.114	0.099	0.093	0.106	-0.33	-0.44	-0.22	**<0.001**	0.108	0.103	0.114	0.099	0.093	0.106	-0.28	-0.39	-0.18	**<0.001**	**<0.001**	0.451	0.549
S VLDL FC, mmol/L	0.071	0.067	0.075	0.065	0.060	0.069	-0.37	-0.48	-0.26	**<0.001**	0.071	0.067	0.075	0.065	0.060	0.069	-0.34	-0.44	-0.24	**<0.001**	**<0.001**	0.510	0.726
S VLDL TAG, mmol/L	0.195	0.184	0.207	0.177	0.167	0.188	-0.32	-0.46	-0.18	**<0.001**	0.195	0.184	0.207	0.177	0.167	0.188	-0.08	-0.22	0.05	0.242	**<0.001**	0.579	**0.018**
Small LDL																							
S LDL P, nmol/L	205.1	197.0	213.5	187.5	179.3	196.1	-0.44	-0.54	-0.34	**<0.001**	201.0	193.0	209.3	187.4	180.1	196.1	-0.38	-0.48	-0.29	**<0.001**	**<0.001**	0.600	0.436
S LDL L, mmol/L	0.349	0.334	0.364	0.317	0.301	0.332	-0.44	-0.53	-0.34	**<0.001**	0.349	0.334	0.364	0.317	0.301	0.332	-0.40	-0.49	-0.31	**<0.001**	**<0.001**	0.631	0.595
S LDL PL, mmol/L	0.109	0.105	0.114	0.101	0.096	0.106	-0.39	-0.48	-0.29	**<0.001**	0.109	0.105	0.114	0.101	0.096	0.106	-0.41	-0.50	-0.32	**<0.001**	**<0.001**	0.615	0.744
S LDL C, mmol/L	0.222	0.212	0.232	0.200	0.190	0.211	-0.45	-0.55	-0.35	**<0.001**	0.222	0.212	0.232	0.200	0.190	0.211	-0.40	-0.50	-0.30	**<0.001**	**<0.001**	0.652	0.479
S LDL CE, mmol/L	0.160	0.153	0.168	0.144	0.136	0.151	-0.46	-0.56	-0.36	**<0.001**	0.160	0.153	0.168	0.144	0.136	0.151	-0.38	-0.48	-0.28	**<0.001**	**<0.001**	0.651	0.270
S LDL FC, mmol/L	0.061	0.059	0.064	0.056	0.053	0.059	-0.39	-0.50	-0.29	**<0.001**	0.061	0.059	0.064	0.056	0.053	0.059	-0.43	-0.53	-0.32	**<0.001**	**<0.001**	0.680	0.684
S LDL TAG, mmol/L	0.017	0.016	0.018	0.015	0.015	0.016	-0.35	-0.47	-0.23	**<0.001**	0.017	0.016	0.018	0.015	0.015	0.016	-0.18	-0.30	-0.07	**0.002**	**<0.001**	0.590	**0.044**
Very large HDL																							
XL HDL P, nmol/L	194.0	183.8	204.7	190.5	180.1	201.4	-0.05	-0.15	0.05	0.336	204.9	193.5	217.0	192.4	183.1	201.4	-0.17	-0.27	-0.08	**<0.001**	**0.002**	0.246	0.080
XL HDL L, mmol/L	0.126	0.117	0.136	0.132	0.123	0.141	0.11	0.01	0.21	**0.039**	0.126	0.117	0.136	0.132	0.123	0.141	-0.08	-0.18	0.01	0.086	0.758	0.292	**0.008**
XL HDL PL, mmol/L	0.050	0.043	0.058	0.057	0.052	0.062	0.13	0.06	0.21	**0.001**	0.050	0.043	0.058	0.057	0.052	0.062	-0.01	-0.09	0.06	0.753	**0.028**	0.268	**0.009**

Table S2. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Very large HDL																							
XL HDL C, mmol/L	0.067	0.063	0.071	0.068	0.064	0.072	0.06	-0.03	0.16	0.203	0.067	0.063	0.071	0.068	0.064	0.072	-0.14	-0.23	-0.04	**0.005**	0.289	0.263	**0.004**
XL HDL CE, mmol/L	0.045	0.042	0.049	0.047	0.043	0.050	0.05	-0.04	0.14	0.253	0.045	0.042	0.049	0.047	0.043	0.050	-0.11	-0.20	-0.03	**0.012**	0.352	0.214	**0.010**
XL HDL FC, mmol/L	0.021	0.020	0.022	0.021	0.021	0.022	0.12	0.01	0.24	**0.040**	0.021	0.020	0.022	0.021	0.021	0.022	-0.17	-0.29	-0.06	**0.003**	0.569	0.574	**<0.001**
XL HDL TAG, mmol/L	0.006	0.006	0.007	0.006	0.006	0.006	-0.32	-0.46	-0.18	**<0.001**	0.006	0.006	0.007	0.006	0.006	0.006	-0.19	-0.32	-0.05	**0.007**	**<0.001**	0.996	0.179
Medium HDL																							
M HDL P, µmol/L	3.28	3.14	3.43	3.09	2.94	3.25	-0.24	-0.34	-0.14	**<0.001**	3.36	3.21	3.52	3.24	3.10	3.25	-0.11	-0.21	-0.02	**0.021**	**<0.001**	0.102	0.079
M HDL L, mmol/L	0.894	0.860	0.931	0.852	0.816	0.890	-0.22	-0.33	-0.12	**<0.001**	0.894	0.860	0.931	0.852	0.816	0.890	-0.11	-0.21	-0.01	**0.036**	**<0.001**	0.124	0.120
M HDL PL, mmol/L	0.428	0.413	0.444	0.409	0.393	0.425	-0.22	-0.33	-0.12	**<0.001**	0.428	0.413	0.444	0.409	0.393	0.425	-0.11	-0.21	-0.01	**0.039**	**<0.001**	0.166	0.125
M HDL C, mmol/L	0.414	0.395	0.434	0.396	0.377	0.416	-0.18	-0.28	-0.07	**0.001**	0.414	0.395	0.434	0.396	0.377	0.416	-0.10	-0.19	0.00	0.053	**<0.001**	0.098	0.273
M HDL CE, mmol/L	0.339	0.323	0.356	0.326	0.310	0.343	-0.15	-0.26	-0.05	**0.003**	0.339	0.323	0.356	0.326	0.310	0.343	-0.08	-0.18	0.02	0.123	**0.002**	0.095	0.283
M HDL FC, mmol/L	0.075	0.071	0.079	0.070	0.066	0.074	-0.25	-0.36	-0.15	**<0.001**	0.075	0.071	0.079	0.070	0.066	0.074	-0.17	-0.27	-0.07	**0.001**	**<0.001**	0.132	0.263
M HDL TAG, mmol/L	0.050	0.047	0.052	0.046	0.044	0.048	-0.31	-0.48	-0.13	**0.001**	0.050	0.047	0.052	0.046	0.044	0.048	-0.04	-0.20	0.13	0.670	**0.005**	0.801	**0.027**
Small HDL																							
S HDL P, µmol/L	9.96	9.75	10.18	9.35	9.14	9.57	-0.54	-0.68	-0.41	**<0.001**	9.92	9.70	10.14	9.58	9.35	9.57	-0.31	-0.44	-0.18	**<0.001**	**<0.001**	0.495	**0.014**
S HDL L, mmol/L	1.164	1.138	1.190	1.096	1.070	1.122	-0.51	-0.65	-0.38	**<0.001**	1.164	1.138	1.190	1.096	1.070	1.122	-0.26	-0.39	-0.13	**<0.001**	**<0.001**	0.442	**0.007**
S HDL PL, mmol/L	0.656	0.641	0.671	0.620	0.605	0.635	-0.48	-0.61	-0.34	**<0.001**	0.656	0.641	0.671	0.620	0.605	0.635	-0.22	-0.35	-0.09	**0.001**	**<0.001**	0.412	**0.008**
S HDL C, mmol/L	0.451	0.441	0.461	0.424	0.414	0.435	-0.52	-0.65	-0.38	**<0.001**	0.451	0.441	0.461	0.424	0.414	0.435	-0.30	-0.43	-0.17	**<0.001**	**<0.001**	0.398	**0.027**
S HDL CE, mmol/L	0.334	0.327	0.341	0.316	0.308	0.323	-0.48	-0.62	-0.35	**<0.001**	0.334	0.327	0.341	0.316	0.308	0.323	-0.27	-0.40	-0.14	**<0.001**	**<0.001**	0.432	**0.029**
S HDL FC, mmol/L	0.117	0.114	0.120	0.109	0.106	0.111	-0.55	-0.68	-0.42	**<0.001**	0.117	0.114	0.120	0.109	0.106	0.111	-0.35	-0.47	-0.22	**<0.001**	**<0.001**	0.371	**0.031**
S HDL TAG, mmol/L	0.055	0.053	0.058	0.051	0.049	0.053	-0.35	-0.49	-0.20	**<0.001**	0.055	0.053	0.058	0.051	0.049	0.053	-0.05	-0.19	0.09	0.444	**<0.001**	0.528	**0.005**
Relative lipoprotein lipid concentrations																							
Very large VLDL																							
XL VLDL PL %	18.5	18.3	18.8	18.7	18.5	19.0	0.16	-0.02	0.33	0.075	18.5	18.3	18.7	18.5	18.3	18.7	-0.03	-0.20	0.14	0.749	0.286	0.365	0.132
XL VLDL C %	24.4	23.7	25.0	25.1	24.2	25.9	0.16	-0.01	0.33	0.067	25.1	24.4	25.9	24.9	24.2	25.6	-0.07	-0.23	0.10	0.425	0.438	0.395	0.061
XL VLDL CE %	13.2	12.7	13.7	13.5	12.9	14.2	0.10	-0.07	0.27	0.242	13.7	13.1	14.3	13.5	13.0	14.1	-0.08	-0.24	0.09	0.364	0.826	0.351	0.141
XL VLDL FC %	11.2	11.0	11.3	11.4	11.2	11.6	0.27	0.09	0.46	**0.003**	11.3	11.1	11.5	11.3	11.1	11.5	0.00	-0.18	0.17	0.987	**0.034**	0.816	**0.032**
XL VLDL TAG %	56.6	55.8	57.5	55.7	54.6	56.7	-0.18	-0.36	-0.01	**0.040**	55.8	54.9	56.8	56.2	55.3	57.1	0.09	-0.08	0.26	0.290	0.446	0.667	**0.027**
Medium VLDL																							
M VLDL PL %	21.5	21.2	21.8	21.5	21.1	21.9	0.02	-0.12	0.17	0.774	21.7	21.4	21.9	21.4	21.1	21.6	-0.21	-0.35	-0.07	**0.003**	0.066	0.791	**0.025**
M VLDL C %	26.5	25.5	27.5	27.1	25.9	28.3	0.07	-0.07	0.22	0.322	27.4	26.3	28.4	26.5	25.4	27.6	-0.16	-0.30	-0.02	**0.023**	0.386	0.713	**0.022**
M VLDL CE %	13.4	12.6	14.2	13.9	13.0	14.8	0.08	-0.06	0.22	0.267	14.0	13.2	14.9	13.4	12.6	14.3	-0.13	-0.26	0.01	0.066	0.637	0.714	**0.039**
M VLDL FC %	13.0	12.7	13.3	13.1	12.7	13.4	0.04	-0.10	0.19	0.564	13.2	12.9	13.5	12.9	12.6	13.1	-0.22	-0.36	-0.08	**0.002**	0.084	0.852	**0.011**
M VLDL TAG %	51.1	49.9	52.4	50.3	48.8	51.9	-0.10	-0.26	0.06	0.224	49.9	48.6	51.2	51.3	50.0	52.6	0.20	0.05	0.35	**0.010**	0.361	0.785	**0.008**

Table S2. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Small VLDL																							
S VLDL PL %	23.8	23.5	24.1	23.8	23.5	24.1	-0.02	-0.17	0.12	0.729	24.0	23.7	24.3	23.6	23.3	23.9	-0.28	-0.41	-0.14	**<0.001**	**0.003**	0.938	**0.011**
S VLDL C %	36.2	35.4	37.0	36.2	35.3	37.2	-0.02	-0.17	0.13	0.821	36.6	35.8	37.3	35.7	35.0	36.5	-0.22	-0.36	-0.07	**0.003**	**0.027**	0.961	0.059
S VLDL CE %	21.8	21.3	22.4	21.9	21.3	22.6	0.02	-0.14	0.17	0.828	22.0	21.5	22.5	21.6	21.1	22.1	-0.14	-0.29	0.00	0.053	0.237	0.888	0.135
S VLDL FC %	14.4	14.1	14.6	14.3	13.9	14.6	-0.08	-0.22	0.06	0.272	14.6	14.3	14.9	14.1	13.8	14.4	-0.32	-0.46	-0.19	**<0.001**	**<0.001**	0.903	**0.012**
S VLDL TAG %	39.4	38.4	40.4	39.2	38.0	40.5	-0.01	-0.18	0.15	0.871	38.8	37.8	39.8	40.1	39.1	41.2	0.26	0.11	0.42	**0.001**	**0.030**	0.876	**0.016**
Very small VLDL																							
XS VLDL PL %	29.3	29.2	29.4	29.7	29.5	29.8	0.50	0.30	0.69	**<0.001**	29.2	29.0	29.3	29.4	29.3	29.6	0.33	0.14	0.51	**0.001**	**<0.001**	0.059	0.205
XS VLDL C %	51.4	50.7	52.1	51.2	50.6	51.9	-0.08	-0.22	0.05	0.216	51.9	51.3	52.5	50.9	50.3	51.5	-0.30	-0.43	-0.17	**<0.001**	**<0.001**	0.743	**0.025**
XS VLDL CE %	34.9	34.3	35.6	34.8	34.2	35.4	-0.08	-0.21	0.05	0.239	35.5	34.9	36.0	34.6	34.0	35.1	-0.27	-0.40	-0.15	**<0.001**	**<0.001**	0.631	**0.033**
XS VLDL FC %	16.4	16.3	16.5	16.4	16.3	16.5	-0.06	-0.22	0.10	0.462	16.4	16.3	16.5	16.3	16.2	16.4	-0.30	-0.45	-0.14	**<0.001**	**0.002**	0.245	**0.041**
XS VLDL TAG %	19.0	18.4	19.6	18.8	18.2	19.4	-0.03	-0.18	0.12	0.669	18.6	18.1	19.1	19.5	18.9	20.0	0.29	0.15	0.43	**<0.001**	**0.015**	0.817	**0.002**
Medium LDL																							
M LDL PL %	25.9	25.7	26.0	26.0	25.9	26.2	0.24	0.07	0.40	**0.007**	26.0	25.8	26.1	25.9	25.8	26.1	-0.03	-0.20	0.13	0.678	0.092	0.845	**0.024**
M LDL C %	69.6	69.4	69.8	69.3	69.0	69.5	-0.36	-0.52	-0.20	**<0.001**	69.5	69.3	69.7	69.4	69.1	69.6	-0.18	-0.33	-0.02	**0.026**	**<0.001**	0.947	0.109
M LDL CE %	50.7	50.3	51.1	50.2	49.8	50.6	-0.26	-0.42	-0.10	**0.001**	50.3	50.0	50.7	50.4	50.0	50.9	0.03	-0.12	0.18	0.701	**0.036**	0.711	**0.009**
M LDL FC %	18.8	18.5	19.1	19.0	18.7	19.3	0.12	-0.03	0.26	0.116	19.1	18.8	19.4	18.8	18.6	19.1	-0.16	-0.30	-0.02	**0.024**	0.664	0.596	**0.007**
M LDL TAG %	4.5	4.4	4.6	4.6	4.5	4.8	0.24	0.11	0.38	**0.001**	4.5	4.3	4.6	4.6	4.5	4.8	0.30	0.17	0.43	**<0.001**	**<0.001**	0.883	0.563
Small LDL																							
S LDL PL %	31.4	31.1	31.6	31.9	31.6	32.2	0.39	0.23	0.55	**<0.001**	31.6	31.4	31.8	31.7	31.4	32.0	0.08	-0.07	0.23	0.291	**<0.001**	0.966	**0.006**
S LDL C %	63.6	63.4	63.8	63.1	62.8	63.5	-0.43	-0.60	-0.27	**<0.001**	63.5	63.3	63.7	63.3	63.0	63.5	-0.24	-0.40	-0.08	**0.003**	**<0.001**	0.917	0.106
S LDL CE %	45.9	45.6	46.3	45.4	45.0	45.7	-0.36	-0.52	-0.19	**<0.001**	45.7	45.4	46.0	45.6	45.2	45.9	-0.06	-0.21	0.09	0.445	**<0.001**	0.968	**0.010**
S LDL FC %	17.6	17.4	17.9	17.7	17.5	18.0	0.06	-0.10	0.22	0.436	17.8	17.5	18.0	17.6	17.4	17.9	-0.15	-0.31	0.00	**0.046**	0.413	0.871	0.053
S LDL TAG %	4.9	4.7	5.0	4.9	4.7	5.0	0.02	-0.12	0.17	0.745	4.8	4.6	4.9	4.9	4.7	5.1	0.23	0.09	0.36	**0.002**	**0.016**	0.783	0.051
Very large HDL																							
XL HDL PL %	39.4	36.1	43.1	43.1	42.0	44.1	0.14	0.08	0.21	**<0.001**	41.7	40.3	43.1	42.4	41.1	43.8	0.05	-0.01	0.12	0.110	**<0.001**	0.363	0.052
XL HDL C %	52.8	52.1	53.6	51.8	51.1	52.5	-0.25	-0.38	-0.13	**<0.001**	52.5	51.6	53.4	52.0	51.2	52.9	-0.17	-0.28	-0.05	**0.005**	**<0.001**	0.650	0.322
XL HDL CE %	36.0	35.4	36.6	35.4	35.0	35.8	-0.20	-0.33	-0.08	**0.002**	36.3	35.7	36.8	35.9	35.4	36.4	-0.14	-0.26	-0.02	**0.020**	**<0.001**	0.279	0.495
XL HDL FC %	16.5	15.8	17.2	16.3	15.7	16.9	-0.06	-0.16	0.04	0.215	16.0	15.3	16.6	16.0	15.4	16.6	-0.03	-0.13	0.06	0.459	0.160	0.154	0.702
XL HDL TAG %	5.1	4.6	5.6	4.4	4.1	4.8	-0.29	-0.41	-0.17	**<0.001**	4.6	4.2	5.0	4.5	4.2	4.9	-0.04	-0.16	0.08	0.504	**<0.001**	0.384	**0.004**
Large HDL																							
L HDL PL %	48.9	44.8	53.3	51.2	50.7	51.7	0.10	-0.03	0.22	0.143	50.9	50.3	51.4	50.9	50.5	51.4	0.00	-0.12	0.13	0.967	0.281	0.433	0.308
L HDL C %	39.9	35.9	44.3	43.1	41.9	44.4	0.14	0.01	0.27	**0.031**	43.2	42.1	44.4	43.5	42.6	44.4	0.01	-0.11	0.14	0.834	0.090	0.136	0.161

Table S2. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Large HDL																							
L HDL CE %	29.5	25.5	34.1	32.9	31.9	34.0	0.17	0.02	0.32	**0.024**	32.8	31.8	33.9	33.4	32.6	34.2	0.03	-0.12	0.17	0.707	0.061	0.128	0.176
L HDL FC %	9.4	8.3	10.6	10.1	9.8	10.4	0.15	0.00	0.29	**0.044**	10.3	10.1	10.5	10.1	9.9	10.3	-0.03	-0.18	0.11	0.624	0.272	0.209	0.075
L HDL TAG %	5.5	4.9	6.1	4.8	4.4	5.3	-0.24	-0.39	-0.09	**0.002**	4.8	4.4	5.3	4.8	4.4	5.3	0.00	-0.14	0.15	0.964	**0.028**	0.282	**0.024**

Values are geometric means with 95% confidence intervals. The differences in fasting plasma metabolites between the diets were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates.

Table S3. Postprandial plasma metabolites (AUC) at week 0 and 12 upon LFHP or HMUFA diet

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Triglycerides																							
Total TAG, mmol/L	121.0	113.5	128.9	108.3	101.0	116.1	-0.31	-0.44	-0.17	**<0.001**	110.4	103.2	118.2	109.6	102.2	117.5	-0.05	-0.18	0.07	0.409	**<0.001**	0.267	**0.007**
VLDL TAG, mmol/L	95.7	89.0	102.9	84.8	78.2	92.0	-0.29	-0.43	-0.16	**<0.001**	85.7	79.2	92.7	85.3	78.6	92.6	-0.04	-0.16	0.09	0.539	**<0.001**	0.223	**0.007**
LDL TAG, mmol/L	9.4	9.0	9.8	8.6	8.2	9.1	-0.33	-0.45	-0.21	**<0.001**	9.1	8.7	9.5	8.9	8.5	9.3	-0.18	-0.28	-0.07	**0.002**	**<0.001**	0.608	0.060
HDL TAG, mmol/L	9.1	8.7	9.5	8.4	8.1	8.9	-0.32	-0.48	-0.15	**<0.001**	8.8	8.4	9.2	8.7	8.3	9.1	0.00	-0.15	0.15	0.976	**0.007**	0.902	**0.006**
Phospholipids																							
Total PL, mmol/L	188	183	193	175	170	181	-0.47	-0.59	-0.34	**<0.001**	188	183	193	178	173	183	-0.39	-0.50	-0.28	**<0.001**	**<0.001**	0.849	0.380
VLDL PL, mmol/L	40	37	42	35	33	38	-0.34	-0.46	-0.22	**<0.001**	37	35	39	35	33	38	-0.17	-0.28	-0.06	**0.003**	**<0.001**	0.263	**0.034**
LDL PL, mmol/L	43	41	45	38	36	41	-0.48	-0.59	-0.37	**<0.001**	42	41	44	39	38	41	-0.42	-0.52	-0.32	**<0.001**	**<0.001**	0.685	0.452
HDL PL, mmol/L	84	82	87	82	79	85	-0.15	-0.25	-0.05	**0.002**	87	84	90	84	81	87	-0.15	-0.24	-0.07	**0.001**	**<0.001**	0.136	0.964
Total lipids																							
Total L, mmol/L	621	600	643	566	544	590	-0.49	-0.61	-0.37	**<0.001**	610	590	630	576	557	596	-0.36	-0.47	-0.25	**<0.001**	**<0.001**	0.759	0.114
VLDL L, mmol/L	190	179	202	169	158	182	-0.33	-0.46	-0.21	**<0.001**	175	164	187	170	159	182	-0.12	-0.24	-0.01	**0.035**	**<0.001**	0.251	**0.016**
LDL L, mmol/L	179	171	188	159	150	168	-0.50	-0.61	-0.39	**<0.001**	176	168	183	163	156	170	-0.41	-0.51	-0.31	**<0.001**	**<0.001**	0.740	0.208
HDL L, mmol/L	161	156	167	156	150	162	-0.17	-0.26	-0.08	**<0.001**	167	161	173	161	155	167	-0.17	-0.26	-0.08	**<0.001**	**<0.001**	0.109	0.999
Lipoprotein particle sizes																							
VLDL size, nm	2428	2415	2441	2416	2401	2430	-0.14	-0.27	-0.02	**0.024**	2408	2394	2423	2418	2402	2434	0.12	0.01	0.24	**0.031**	0.831	0.280	**0.002**
LDL size, nm	1432	1431	1433	1432	1431	1433	0.11	-0.06	0.28	0.205	1433	1432	1434	1431	1430	1432	-0.27	-0.43	-0.12	**0.001**	0.166	0.718	**0.001**
HDL size, nm	571	569	573	572	570	574	0.14	0.05	0.22	**0.001**	573	571	576	573	571	574	-0.02	-0.10	0.06	0.597	**0.045**	0.129	**0.007**

Table S3. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Other lipids																							
Phosphoglyc, mmol/L	147	143	152	137	132	142	-0.43	-0.55	-0.31	**<0.001**	147	143	152	140	136	145	-0.32	-0.43	-0.21	**<0.001**	**<0.001**	0.660	0.193
TAG/PG, ratio	49	47	52	47	45	50	-0.13	-0.25	-0.01	**0.040**	45	42	48	47	44	50	0.10	-0.01	0.21	0.085	0.725	0.120	**0.008**
Cholines, mmol/L	163	158	167	152	147	157	-0.44	-0.56	-0.32	**<0.001**	163	159	168	156	151	160	-0.35	-0.46	-0.24	**<0.001**	**<0.001**	0.588	0.261
Phosphatidylc, mmol/L	138	134	142	127	123	132	-0.46	-0.59	-0.34	**<0.001**	138	134	142	131	127	135	-0.32	-0.43	-0.21	**<0.001**	**<0.001**	0.553	0.084
Sphingomyelins, mmol/L	28	27	28	26	25	27	-0.43	-0.54	-0.31	**<0.001**	28	27	29	26	26	27	-0.46	-0.57	-0.36	**<0.001**	**<0.001**	0.636	0.649
Fatty acids																							
Total FA, mmol/L	875	843	907	813	780	848	-0.36	-0.49	-0.24	**<0.001**	850	821	881	824	793	856	-0.19	-0.31	-0.08	**0.001**	**<0.001**	0.587	**0.046**
Omega 3, mmol/L	29	26	31	29	26	32	-0.07	-0.20	0.05	0.255	28	26	30	25	23	28	-0.26	-0.37	-0.14	**<0.001**	**<0.001**	0.182	**0.032**
Omega 6, mmol/L	306	298	314	290	281	299	-0.39	-0.52	-0.27	**<0.001**	304	296	312	293	284	301	-0.31	-0.42	-0.19	**<0.001**	**<0.001**	0.953	0.321
PUFA, mmol/L	337	327	347	320	309	331	-0.35	-0.47	-0.23	**<0.001**	333	324	343	319	310	329	-0.31	-0.42	-0.20	**<0.001**	**<0.001**	0.687	0.623
MUFA, mmol/L	236	225	247	216	205	227	-0.32	-0.44	-0.20	**<0.001**	224	213	234	225	214	236	-0.01	-0.13	0.10	0.807	**<0.001**	0.624	**<0.001**
SFA, mmol/L	301	289	313	276	264	289	-0.38	-0.52	-0.25	**<0.001**	292	281	303	279	268	291	-0.24	-0.36	-0.11	**<0.001**	**<0.001**	0.509	0.115
LA, mmol/L	245	237	253	228	219	237	-0.41	-0.54	-0.28	**<0.001**	242	234	250	232	224	240	-0.26	-0.38	-0.14	**<0.001**	**<0.001**	0.973	0.101
DHA, mmol/L	12	11	13	12	11	13	-0.15	-0.29	-0.01	**0.033**	12	11	13	11	10	12	-0.33	-0.45	-0.20	**<0.001**	**<0.001**	0.368	0.065
Fatty acid ratios																							
Omega 3 %	198	185	212	214	199	229	0.13	-0.01	0.26	0.068	198	187	209	184	171	198	-0.21	-0.33	-0.09	**0.001**	0.371	0.158	**<0.001**
Omega 6 %	2107	2076	2140	2148	2113	2184	0.20	0.05	0.34	**0.008**	2153	2123	2183	2138	2107	2170	-0.07	-0.21	0.06	0.275	0.216	0.248	**0.007**
PUFA %	2317	2286	2349	2373	2340	2406	0.25	0.11	0.39	**0.001**	2359	2327	2390	2333	2302	2365	-0.14	-0.27	-0.01	**0.031**	0.283	0.609	**<0.001**
MUFA %	1613	1589	1637	1588	1562	1614	-0.13	-0.25	-0.01	**0.041**	1576	1552	1600	1632	1607	1658	0.40	0.29	0.52	**<0.001**	**0.002**	0.825	**<0.001**
SFA %	2059	2044	2073	2029	2012	2047	-0.31	-0.49	-0.12	**0.001**	2054	2040	2068	2024	2009	2039	-0.38	-0.55	-0.21	**<0.001**	**<0.001**	0.430	0.565
LA %	1685	1658	1713	1688	1658	1718	-0.02	-0.17	0.13	0.828	1712	1689	1736	1698	1672	1724	-0.10	-0.24	0.04	0.151	0.258	0.189	0.416
DHA %	84	79	89	88	83	93	0.09	-0.07	0.24	0.272	85	81	90	80	75	86	-0.22	-0.36	-0.08	**0.003**	0.216	0.484	**0.005**
PUFA/MUFA, ratio	86	84	89	90	87	93	0.19	0.06	0.32	**0.004**	90	88	93	86	84	88	-0.29	-0.41	-0.17	**<0.001**	0.259	0.729	**<0.001**
Omega 6/Omega 3, ratio	640	594	690	604	559	653	-0.08	-0.22	0.06	0.248	655	617	695	698	647	753	0.18	0.05	0.31	**0.005**	0.293	0.121	**0.006**
Amino acids																							
Alanine, mmol/L	20.4	19.9	21.1	20.7	20.0	21.5	0.05	-0.13	0.22	0.585	21.1	20.5	21.7	21.9	21.2	22.5	0.22	0.05	0.38	**0.009**	**0.030**	**0.029**	0.170
Glutamine, mmol/L	36.4	35.2	37.6	35.6	34.3	37.0	-0.30	-0.49	-0.11	**0.003**	35.8	34.6	36.9	36.2	35.0	37.5	0.01	-0.17	0.19	0.917	**0.031**	0.697	**0.022**
Glycine, mmol/L	9.1	8.5	9.6	9.6	9.0	10.3	0.12	-0.01	0.24	0.063	9.9	9.3	10.6	11.1	10.4	11.9	0.29	0.18	0.41	**<0.001**	**<0.001**	**0.006**	**0.044**
Histidine, mmol/L	4.7	4.6	4.8	4.7	4.5	4.8	-0.09	-0.31	0.14	0.454	4.7	4.6	4.7	4.7	4.6	4.8	0.10	-0.11	0.31	0.348	0.930	0.756	0.236
Branched-chain amino acids																							
Total BCAA, mmol/L	23.7	23.1	24.3	22.8	22.1	23.5	-0.22	-0.36	-0.07	**0.003**	23.9	23.3	24.5	24.0	23.4	24.6	0.00	-0.13	0.13	0.988	**0.030**	0.121	**0.028**
Isoleucine, mmol/L	3.6	3.5	3.7	3.4	3.3	3.6	-0.17	-0.33	0.00	**0.047**	3.5	3.4	3.6	3.6	3.5	3.7	0.11	-0.04	0.26	0.166	0.596	0.658	**0.017**
Leucine, mmol/L	6.8	6.6	7.0	6.5	6.3	6.8	-0.17	-0.32	-0.03	**0.017**	6.8	6.6	7.0	6.9	6.7	7.1	0.04	-0.09	0.17	0.572	0.168	0.176	**0.033**
Valine, mmol/L	13.3	13.0	13.6	12.8	12.4	13.2	-0.25	-0.40	-0.11	**0.001**	13.5	13.2	13.8	13.5	13.2	13.8	-0.06	-0.19	0.08	0.415	**0.003**	0.050	0.053

Table S3. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Ketone bodies																							
3-Hydroxybutyrate, mmol/L	2.8	2.6	3.1	2.2	2.0	2.5	-0.50	-0.70	-0.29	**<0.001**	3.3	3.0	3.5	3.0	2.8	3.3	-0.21	-0.39	-0.02	**0.030**	**<0.001**	**<0.001**	**0.040**
Acetate, mmol/L	1.7	1.7	1.8	1.7	1.5	1.9	-0.16	-0.42	0.10	0.222	1.8	1.7	1.9	1.6	1.5	1.7	-0.39	-0.63	-0.14	**0.002**	**0.003**	0.765	0.220
Acetoacetate, mmol/L	1.9	1.8	2.1	1.8	1.6	1.9	-0.13	-0.32	0.06	0.174	2.2	2.1	2.4	2.1	2.0	2.3	-0.12	-0.29	0.06	0.184	0.058	**<0.001**	0.923
Acetone, mmol/L	1.0	0.9	1.0	1.0	0.9	1.0	-0.04	-0.22	0.13	0.647	1.0	1.0	1.0	1.0	1.0	1.1	0.01	-0.15	0.17	0.893	0.807	**0.011**	0.670
Lipoprotein subclasses																							
Chylomicrons and extremely large VLDL																							
XXL VLDL P, µmol/L	0.17	0.16	0.19	0.16	0.14	0.17	-0.13	-0.28	0.02	0.094	0.14	0.13	0.16	0.15	0.13	0.17	0.09	-0.05	0.23	0.189	0.733	0.072	**0.034**
XXL VLDL L, mmol/L	23.6	21.4	26.1	21.6	19.4	24.0	-0.12	-0.28	0.03	0.118	23.6	21.4	26.1	21.6	19.4	24.0	0.08	-0.06	0.22	0.253	0.706	0.071	0.055
XXL VLDL PL, mmol/L	3.1	2.8	3.5	2.9	2.6	3.2	-0.09	-0.25	0.07	0.263	3.1	2.8	3.5	2.9	2.6	3.2	0.13	-0.01	0.28	0.077	0.702	0.060	**0.044**
XXL VLDL C, mmol/L	4.5	4.1	4.9	4.0	3.6	4.5	-0.15	-0.30	-0.01	**0.042**	4.5	4.1	4.9	4.0	3.6	4.5	0.08	-0.05	0.22	0.237	0.484	0.100	**0.022**
XXL VLDL CE, mmol/L	2.4	2.1	2.6	2.1	1.9	2.3	-0.20	-0.34	-0.06	**0.006**	2.4	2.1	2.6	2.1	1.9	2.3	0.05	-0.07	0.18	0.410	0.141	0.126	**0.010**
XXL VLDL FC, mmol/L	2.1	1.9	2.3	1.9	1.8	2.1	-0.10	-0.26	0.06	0.207	2.1	1.9	2.3	1.9	1.8	2.1	0.11	-0.03	0.26	0.125	0.909	0.083	**0.050**
XXL VLDL TAG, mmol/L	16.0	14.4	17.7	14.5	13.1	16.2	-0.12	-0.28	0.03	0.128	16.0	14.4	17.7	14.5	13.1	16.2	0.07	-0.07	0.22	0.314	0.661	0.068	0.072
Very large VLDL																							
XL VLDL P, µmol/L	0.39	0.36	0.43	0.34	0.31	0.38	-0.28	-0.41	-0.14	**<0.001**	0.34	0.31	0.37	0.34	0.31	0.38	-0.02	-0.15	0.10	0.689	**0.001**	0.175	**0.006**
XL VLDL L, mmol/L	23.2	21.3	25.2	20.2	18.3	22.2	-0.28	-0.41	-0.15	**<0.001**	23.2	21.3	25.2	20.2	18.3	22.2	-0.03	-0.15	0.09	0.623	**0.001**	0.184	**0.007**
XL VLDL PL, mmol/L	4.3	3.9	4.7	3.7	3.4	4.1	-0.26	-0.39	-0.13	**<0.001**	4.3	3.9	4.7	3.7	3.4	4.1	-0.03	-0.15	0.09	0.659	**0.002**	0.161	**0.011**
XL VLDL C, mmol/L	5.1	4.7	5.5	4.5	4.1	4.9	-0.31	-0.43	-0.19	**<0.001**	5.1	4.7	5.5	4.5	4.1	4.9	-0.09	-0.20	0.02	0.124	**<0.001**	0.223	**0.008**
XL VLDL CE, mmol/L	2.6	2.4	2.8	2.3	2.1	2.5	-0.34	-0.45	-0.23	**<0.001**	2.6	2.4	2.8	2.3	2.1	2.5	-0.14	-0.24	-0.03	**0.009**	**<0.001**	0.278	**0.008**
XL VLDL FC, mmol/L	2.5	2.3	2.7	2.2	2.0	2.4	-0.26	-0.39	-0.13	**<0.001**	2.5	2.3	2.7	2.2	2.0	2.4	-0.04	-0.15	0.08	0.554	**0.001**	0.180	**0.012**
XL VLDL TAG, mmol/L	13.8	12.6	15.0	11.9	10.8	13.2	-0.27	-0.41	-0.13	**<0.001**	13.8	12.6	15.0	11.9	10.8	13.2	-0.01	-0.14	0.12	0.859	**0.003**	0.185	**0.007**
Large VLDL																							
L VLDL P, µmol/L	1.12	1.04	1.21	0.98	0.90	1.07	-0.31	-0.44	-0.18	**<0.001**	0.99	0.91	1.08	0.99	0.91	1.08	-0.05	-0.17	0.07	0.379	**<0.001**	0.227	**0.005**
L VLDL L, mmol/L	37.0	34.2	39.9	32.2	29.4	35.2	-0.32	-0.45	-0.19	**<0.001**	37.0	34.2	39.9	32.2	29.4	35.2	-0.07	-0.19	0.05	0.223	**<0.001**	0.231	**0.006**
L VLDL PL, mmol/L	7.2	6.6	7.8	6.3	5.7	6.9	-0.30	-0.43	-0.17	**<0.001**	7.2	6.6	7.8	6.3	5.7	6.9	-0.05	-0.17	0.07	0.418	**<0.001**	0.204	**0.005**
L VLDL C, mmol/L	9.4	8.8	10.2	8.3	7.6	9.1	-0.31	-0.43	-0.19	**<0.001**	9.4	8.8	10.2	8.3	7.6	9.1	-0.09	-0.20	0.02	0.103	**<0.001**	0.251	**0.009**
L VLDL CE, mmol/L	4.6	4.3	5.0	4.1	3.8	4.5	-0.31	-0.43	-0.20	**<0.001**	4.6	4.3	5.0	4.1	3.8	4.5	-0.12	-0.23	-0.01	**0.027**	**<0.001**	0.303	**0.018**
L VLDL FC, mmol/L	4.8	4.5	5.2	4.2	3.9	4.6	-0.31	-0.43	-0.18	**<0.001**	4.8	4.5	5.2	4.2	3.9	4.6	-0.06	-0.18	0.05	0.270	**<0.001**	0.217	**0.005**
L VLDL TAG, mmol/L	20.3	18.7	21.9	17.5	16.0	19.2	-0.33	-0.46	-0.19	**<0.001**	20.3	18.7	21.9	17.5	16.0	19.2	-0.07	-0.20	0.05	0.245	**<0.001**	0.243	**0.007**
Medium VLDL																							
M VLDL P, µmol/L	2.94	2.77	3.13	2.60	2.41	2.80	-0.39	-0.50	-0.29	**<0.001**	2.77	2.61	2.93	2.63	2.48	2.79	-0.24	-0.34	-0.13	**<0.001**	**<0.001**	0.373	**0.036**
M VLDL L, mmol/L	51.4	48.4	54.7	45.3	42.0	48.7	-0.40	-0.52	-0.29	**<0.001**	51.4	48.4	54.7	45.3	42.0	48.7	-0.21	-0.32	-0.11	**<0.001**	**<0.001**	0.368	**0.018**
M VLDL PL, mmol/L	10.8	10.1	11.6	9.5	8.7	10.3	-0.40	-0.50	-0.29	**<0.001**	10.8	10.1	11.6	9.5	8.7	10.3	-0.26	-0.36	-0.16	**<0.001**	**<0.001**	0.419	0.062

Table S3. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Medium VLDL																							
M VLDL C, mmol/L	12.5	11.7	13.4	11.0	10.1	12.0	-0.38	-0.48	-0.28	**<0.001**	12.5	11.7	13.4	11.0	10.1	12.0	-0.35	-0.44	-0.26	**<0.001**	**<0.001**	0.612	0.632
M VLDL CE, mmol/L	6.1	5.7	6.6	5.4	4.9	6.0	-0.32	-0.43	-0.22	**<0.001**	6.1	5.7	6.6	5.4	4.9	6.0	-0.33	-0.43	-0.24	**<0.001**	**<0.001**	0.757	0.864
M VLDL FC, mmol/L	6.3	5.9	6.8	5.5	5.1	6.0	-0.40	-0.51	-0.30	**<0.001**	6.3	5.9	6.8	5.5	5.1	6.0	-0.30	-0.40	-0.21	**<0.001**	**<0.001**	0.477	0.176
M VLDL TAG, mmol/L	27.7	25.9	29.6	24.4	22.5	26.4	-0.37	-0.50	-0.23	**<0.001**	27.7	25.9	29.6	24.4	22.5	26.4	-0.10	-0.22	0.03	0.119	**<0.001**	0.326	**0.004**
Small VLDL																							
S VLDL P, µmol/L	2.94	2.80	3.09	2.65	2.50	2.81	-0.40	-0.52	-0.28	**<0.001**	2.78	2.64	2.93	2.69	2.56	2.83	-0.20	-0.31	-0.09	**<0.001**	**<0.001**	0.411	**0.016**
S VLDL L, mmol/L	30.2	28.7	31.7	27.2	25.7	28.9	-0.41	-0.53	-0.30	**<0.001**	28.7	27.3	30.1	27.6	26.2	28.9	-0.23	-0.34	-0.12	**<0.001**	**<0.001**	0.415	**0.024**
S VLDL PL, mmol/L	7.0	6.7	7.4	6.3	5.9	6.7	-0.42	-0.52	-0.31	**<0.001**	6.7	6.4	7.1	6.4	6.1	6.7	-0.30	-0.40	-0.21	**<0.001**	**<0.001**	0.438	0.129
S VLDL C, mmol/L	10.3	9.8	10.9	9.2	8.6	9.9	-0.41	-0.52	-0.30	**<0.001**	10.0	9.5	10.5	9.3	8.9	9.8	-0.33	-0.43	-0.23	**<0.001**	**<0.001**	0.522	0.265
S VLDL CE, mmol/L	6.2	5.9	6.6	5.6	5.2	6.0	-0.39	-0.51	-0.28	**<0.001**	6.0	5.7	6.3	5.6	5.4	5.9	-0.30	-0.40	-0.19	**<0.001**	**<0.001**	0.514	0.209
S VLDL FC, mmol/L	4.1	3.9	4.4	3.6	3.4	3.9	-0.43	-0.53	-0.32	**<0.001**	4.0	3.8	4.2	3.7	3.5	3.9	-0.37	-0.46	-0.27	**<0.001**	**<0.001**	0.541	0.386
S VLDL TAG, mmol/L	12.6	11.9	13.3	11.5	10.8	12.2	-0.34	-0.48	-0.20	**<0.001**	11.8	11.1	12.5	11.7	11.0	12.5	-0.06	-0.19	0.08	0.405	**<0.001**	0.438	**0.004**
Medium LDL																							
M LDL P, mmol/L	22.3	21.1	23.5	19.7	18.4	21.0	-0.45	-0.57	-0.34	**<0.001**	21.1	20.0	22.2	20.1	19.1	21.2	-0.25	-0.35	-0.14	**<0.001**	**<0.001**	0.458	**0.009**
M LDL L, mmol/L	47.5	45.1	50.0	41.5	38.8	44.3	-0.49	-0.59	-0.38	**<0.001**	45.5	43.3	47.8	42.8	40.7	45.1	-0.32	-0.41	-0.22	**<0.001**	**<0.001**	0.574	**0.021**
M LDL PL, mmol/L	12.0	11.5	12.6	10.6	10.0	11.3	-0.49	-0.59	-0.38	**<0.001**	11.7	11.2	12.2	10.9	10.4	11.4	-0.37	-0.47	-0.28	**<0.001**	**<0.001**	0.604	0.109
M LDL C, mmol/L	33.1	31.4	35.0	28.8	26.9	30.9	-0.49	-0.59	-0.38	**<0.001**	31.7	30.1	33.3	29.8	28.2	31.5	-0.30	-0.40	-0.21	**<0.001**	**<0.001**	0.571	**0.015**
M LDL CE, mmol/L	24.6	23.2	26.0	21.3	19.8	22.9	-0.47	-0.58	-0.36	**<0.001**	23.3	22.0	24.6	22.1	20.9	23.4	-0.25	-0.36	-0.15	**<0.001**	**<0.001**	0.512	**0.005**
M LDL FC, mmol/L	8.5	8.1	8.9	7.4	7.0	7.9	-0.50	-0.61	-0.39	**<0.001**	8.4	8.0	8.8	7.6	7.3	8.0	-0.44	-0.54	-0.34	**<0.001**	**<0.001**	0.819	0.425
M LDL TAG, mmol/L	2.2	2.1	2.3	2.0	1.9	2.1	-0.35	-0.46	-0.23	**<0.001**	2.1	2.0	2.2	2.1	2.0	2.2	-0.14	-0.25	-0.03	**0.012**	**<0.001**	0.583	**0.010**
Small LDL																							
S LDL P, mmol/L	12.6	12.1	13.1	11.4	10.9	12.0	-0.43	-0.54	-0.32	**<0.001**	12.2	11.7	12.7	11.6	11.1	12.0	-0.33	-0.43	-0.23	**<0.001**	**<0.001**	0.518	0.158
S LDL L, mmol/L	21.4	20.5	22.4	19.3	18.2	20.4	-0.45	-0.55	-0.35	**<0.001**	20.8	19.9	21.7	19.6	18.8	20.5	-0.34	-0.43	-0.25	**<0.001**	**<0.001**	0.522	0.126
S LDL PL, mmol/L	6.7	6.4	6.9	6.1	5.8	6.4	-0.41	-0.51	-0.32	**<0.001**	6.5	6.3	6.8	6.1	5.9	6.4	-0.38	-0.47	-0.30	**<0.001**	**<0.001**	0.514	0.661
S LDL C, mmol/L	13.6	13.0	14.2	12.1	11.4	12.8	-0.46	-0.57	-0.36	**<0.001**	13.1	12.6	13.7	12.4	11.8	12.9	-0.34	-0.43	-0.25	**<0.001**	**<0.001**	0.559	0.082
S LDL CE, mmol/L	9.9	9.5	10.4	8.8	8.3	9.3	-0.46	-0.57	-0.35	**<0.001**	9.5	9.1	10.0	9.0	8.6	9.5	-0.30	-0.40	-0.20	**<0.001**	**<0.001**	0.533	**0.028**
S LDL FC, mmol/L	3.6	3.5	3.8	3.3	3.1	3.5	-0.44	-0.55	-0.34	**<0.001**	3.6	3.4	3.7	3.3	3.2	3.5	-0.43	-0.53	-0.34	**<0.001**	**<0.001**	0.669	0.870
S LDL TAG, mmol/L	1.2	1.1	1.2	1.1	1.0	1.2	-0.29	-0.41	-0.16	**<0.001**	1.1	1.0	1.2	1.1	1.0	1.2	-0.04	-0.16	0.07	0.443	**<0.001**	0.343	**0.005**
Very large HDL																							
XL HDL P, mmol/L	12.0	11.4	12.6	11.9	11.2	12.6	-0.02	-0.11	0.07	0.677	12.8	12.1	13.6	12.1	11.5	12.7	-0.17	-0.26	-0.09	**<0.001**	**0.003**	0.228	**0.016**
XL HDL L, mmol/L	7.7	7.2	8.3	8.1	7.6	8.7	0.13	0.04	0.22	**0.007**	8.5	7.9	9.2	8.0	7.5	8.6	-0.11	-0.19	-0.02	**0.014**	0.753	0.277	**<0.001**
XL HDL PL, mmol/L	3.1	2.7	3.5	3.6	3.3	3.9	0.17	0.09	0.25	**<0.001**	3.6	3.2	4.0	3.4	3.1	3.8	-0.03	-0.11	0.04	0.375	**0.019**	0.284	**<0.001**
XL HDL C, mmol/L	4.0	3.7	4.2	4.1	3.9	4.4	0.08	-0.01	0.17	0.096	4.4	4.1	4.6	4.1	3.9	4.3	-0.16	-0.24	-0.07	**<0.001**	0.217	0.238	**<0.001**
XL HDL CE, mmol/L	2.7	2.5	2.9	2.8	2.6	3.0	0.07	-0.02	0.15	0.133	3.0	2.8	3.2	2.8	2.6	3.0	-0.13	-0.21	-0.05	**0.001**	0.256	0.203	**0.001**

Table S3. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Very large HDL																							
XL HDL FC, mmol/L	1.3	1.2	1.3	1.3	1.3	1.4	0.15	0.03	0.26	**0.013**	1.3	1.3	1.4	1.3	1.2	1.3	-0.20	-0.30	-0.09	**<0.001**	0.516	0.471	**<0.001**
XL HDL TAG, mmol/L	0.4	0.4	0.5	0.4	0.4	0.4	-0.28	-0.42	-0.14	**<0.001**	0.4	0.4	0.5	0.4	0.4	0.4	-0.08	-0.21	0.05	0.227	**<0.001**	0.890	**0.046**
Large HDL																							
L HDL P, mmol/L	50.1	43.0	58.5	53.1	47.2	59.8	0.09	-0.02	0.20	0.117	58.2	51.5	65.8	55.7	49.9	62.1	-0.04	-0.14	0.06	0.455	0.524	0.162	0.098
L HDL L, mmol/L	25.1	22.4	28.0	26.3	23.8	29.2	0.10	0.01	0.19	**0.033**	28.4	25.6	31.5	27.1	24.6	29.8	-0.05	-0.13	0.03	0.227	0.454	0.168	**0.018**
L HDL PL, mmol/L	12.8	11.3	14.5	13.7	12.5	15.1	0.14	0.02	0.25	**0.024**	14.7	13.3	16.2	14.0	12.8	15.4	-0.05	-0.16	0.06	0.352	0.304	0.157	**0.022**
L HDL C, mmol/L	9.8	8.2	11.8	10.9	9.6	12.4	0.12	0.01	0.22	**0.028**	11.8	10.4	13.5	11.3	10.1	12.7	-0.03	-0.13	0.07	0.597	0.213	0.157	**0.050**
L HDL CE, mmol/L	7.1	5.7	8.8	8.2	7.1	9.4	0.14	0.01	0.26	**0.030**	8.8	7.6	10.1	8.5	7.6	9.6	-0.01	-0.13	0.10	0.810	0.157	0.144	0.081
L HDL FC, mmol/L	2.5	2.1	3.0	2.7	2.4	3.1	0.12	0.02	0.22	**0.020**	3.0	2.7	3.4	2.8	2.5	3.1	-0.06	-0.15	0.04	0.230	0.377	0.168	**0.012**
L HDL TAG, mmol/L	1.5	1.4	1.6	1.4	1.3	1.5	-0.19	-0.33	-0.06	**0.006**	1.5	1.4	1.6	1.5	1.4	1.6	0.01	-0.12	0.13	0.935	**0.047**	0.398	**0.036**
Small HDL																							
S HDL P, mmol/L	597	584	610	558	543	572	-0.57	-0.71	-0.43	**<0.001**	592	580	605	574	561	588	-0.31	-0.44	-0.18	**<0.001**	**<0.001**	0.626	**0.006**
S HDL L, mmol/L	70.7	69.2	72.3	66.5	64.8	68.2	-0.52	-0.66	-0.38	**<0.001**	70.3	68.9	71.7	68.4	66.9	70.0	-0.25	-0.38	-0.12	**<0.001**	**<0.001**	0.557	**0.007**
S HDL PL, mmol/L	40.1	39.3	41.0	37.9	36.9	38.9	-0.47	-0.61	-0.32	**<0.001**	40.0	39.1	40.8	39.0	38.1	39.9	-0.22	-0.35	-0.09	**0.001**	**<0.001**	0.502	**0.014**
S HDL C, mmol/L	26.9	26.3	27.5	25.1	24.5	25.8	-0.55	-0.69	-0.41	**<0.001**	26.8	26.2	27.3	25.9	25.3	26.6	-0.31	-0.44	-0.18	**<0.001**	**<0.001**	0.487	**0.014**
S HDL CE, mmol/L	19.8	19.4	20.2	18.6	18.1	19.1	-0.52	-0.66	-0.38	**<0.001**	19.7	19.3	20.1	19.1	18.7	19.6	-0.28	-0.41	-0.15	**<0.001**	**<0.001**	0.537	**0.013**
S HDL FC, mmol/L	7.1	6.9	7.2	6.5	6.4	6.7	-0.57	-0.70	-0.43	**<0.001**	7.1	6.9	7.2	6.8	6.6	6.9	-0.36	-0.49	-0.24	**<0.001**	**<0.001**	0.419	**0.029**
S HDL TAG, mmol/L	3.7	3.5	3.8	3.4	3.2	3.5	-0.33	-0.48	-0.18	**<0.001**	3.4	3.3	3.6	3.5	3.3	3.6	0.02	-0.12	0.16	0.777	**0.003**	0.402	**0.001**
Relative lipoprotein lipid concentrations																							
Chylomicrons and extremely large VLDL																							
XXL VLDL PL %	797	785	809	820	805	836	0.33	0.14	0.52	**0.001**	793	781	806	821	806	835	0.39	0.21	0.57	**<0.001**	**<0.001**	0.886	0.620
XXL VLDL C %	1172	1144	1202	1161	1131	1193	-0.11	-0.28	0.07	0.221	1186	1159	1213	1194	1168	1220	0.00	-0.16	0.17	0.965	0.390	0.228	0.357
XXL VLDL CE %	628	605	653	600	574	627	-0.28	-0.44	-0.12	**0.001**	627	607	648	629	610	648	-0.06	-0.21	0.10	0.464	**0.003**	0.445	**0.048**
XXL VLDL FC %	540	530	550	556	541	571	0.24	0.05	0.43	**0.013**	554	542	566	562	549	575	0.12	-0.06	0.30	0.194	**0.007**	0.118	0.349
XXL VLDL TAG %	4014	3976	4053	4003	3959	4048	-0.05	-0.23	0.14	0.620	4005	3969	4042	3973	3937	4009	-0.12	-0.30	0.05	0.155	0.182	0.459	0.537
Very large VLDL																							
XL VLDL PL %	1104	1094	1114	1111	1098	1125	0.17	-0.02	0.37	0.086	1101	1092	1110	1103	1094	1111	0.03	-0.15	0.21	0.736	0.137	0.251	0.301
XL VLDL C %	1335	1307	1364	1349	1312	1387	0.05	-0.11	0.21	0.547	1372	1341	1405	1352	1321	1383	-0.17	-0.32	-0.02	**0.026**	0.284	0.314	0.051
XL VLDL CE %	691	669	715	695	667	724	-0.02	-0.18	0.14	0.798	720	695	747	701	677	727	-0.20	-0.35	-0.06	**0.005**	**0.037**	0.283	0.086
XL VLDL FC %	641	634	648	651	641	662	0.26	0.06	0.45	**0.010**	648	641	656	647	640	655	-0.02	-0.20	0.16	0.830	0.080	0.726	**0.042**
XL VLDL TAG %	3548	3515	3582	3522	3474	3571	-0.11	-0.29	0.07	0.224	3510	3474	3547	3533	3497	3568	0.15	-0.02	0.32	0.083	0.775	0.578	**0.039**
Large VLDL																							
L VLDL PL %	1169	1159	1179	1168	1154	1181	0.01	-0.13	0.15	0.886	1155	1144	1166	1169	1159	1178	0.20	0.07	0.33	**0.002**	**0.029**	0.171	**0.047**
L VLDL C %	1540	1515	1565	1566	1533	1599	0.15	0.00	0.31	0.056	1562	1537	1587	1562	1537	1587	-0.06	-0.20	0.09	0.440	0.374	0.591	0.054

Table S3. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Large VLDL																							
L VLDL CE %	754	733	775	774	749	800	0.13	-0.01	0.28	0.063	776	755	798	771	750	793	-0.10	-0.23	0.03	0.148	0.695	0.453	**0.019**
L VLDL FC %	783	777	790	789	780	798	0.17	-0.01	0.36	0.070	783	777	788	788	782	793	0.10	-0.07	0.27	0.231	**0.033**	0.641	0.600
L VLDL TAG %	3281	3252	3311	3254	3214	3294	-0.15	-0.32	0.01	0.064	3273	3245	3301	3262	3235	3289	-0.02	-0.17	0.13	0.829	0.131	0.903	0.222
Medium VLDL																							
M VLDL PL %	1263	1247	1280	1258	1235	1281	-0.02	-0.17	0.12	0.751	1277	1262	1292	1258	1244	1273	-0.24	-0.38	-0.11	**<0.001**	**0.009**	0.697	**0.031**
M VLDL C %	1466	1413	1521	1466	1398	1537	-0.01	-0.14	0.13	0.941	1526	1467	1587	1459	1402	1519	-0.23	-0.36	-0.11	**<0.001**	**0.012**	0.536	**0.016**
M VLDL CE %	718	677	761	721	673	774	0.00	-0.13	0.14	0.956	758	710	809	717	672	764	-0.19	-0.31	-0.07	**0.003**	**0.046**	0.584	**0.038**
M VLDL FC %	741	726	756	737	717	758	-0.03	-0.17	0.11	0.640	757	742	771	734	720	749	-0.29	-0.42	-0.16	**<0.001**	**0.001**	0.598	**0.008**
M VLDL TAG %	3227	3163	3293	3221	3136	3308	-0.02	-0.18	0.13	0.769	3144	3076	3214	3237	3167	3309	0.28	0.13	0.42	**<0.001**	**0.019**	0.532	**0.006**
Small VLDL																							
S VLDL PL %	1400	1382	1417	1392	1370	1414	-0.07	-0.21	0.07	0.305	1412	1395	1430	1386	1370	1403	-0.32	-0.45	-0.19	**<0.001**	**<0.001**	0.918	**0.013**
S VLDL C %	2059	2012	2106	2033	1973	2096	-0.08	-0.23	0.06	0.262	2092	2046	2139	2030	1984	2077	-0.26	-0.39	-0.13	**<0.001**	**0.001**	0.773	0.077
S VLDL CE %	1238	1208	1269	1227	1188	1268	-0.05	-0.20	0.10	0.526	1254	1224	1285	1227	1196	1258	-0.19	-0.32	-0.05	**0.009**	**0.025**	0.823	0.186
S VLDL FC %	820	802	838	805	782	828	-0.14	-0.27	0.00	**0.048**	836	819	854	802	785	820	-0.37	-0.49	-0.24	**<0.001**	**<0.001**	0.710	**0.014**
S VLDL TAG %	2508	2450	2568	2532	2457	2610	0.06	-0.10	0.22	0.445	2460	2401	2520	2553	2493	2614	0.32	0.17	0.46	**<0.001**	**0.001**	0.866	**0.021**
Very small VLDL																							
XS VLDL PL %	1744	1737	1750	1761	1753	1769	0.39	0.20	0.58	**<0.001**	1738	1730	1747	1745	1736	1754	0.16	-0.01	0.34	0.072	**<0.001**	**0.045**	0.078
XS VLDL C %	3037	2995	3080	3024	2980	3069	-0.07	-0.21	0.06	0.262	3076	3039	3114	3012	2973	3050	-0.27	-0.39	-0.15	**<0.001**	**<0.001**	0.549	**0.030**
XS VLDL CE %	2071	2031	2112	2063	2024	2103	-0.05	-0.18	0.07	0.412	2109	2074	2145	2054	2018	2090	-0.24	-0.36	-0.12	**<0.001**	**0.001**	0.500	**0.035**
XS VLDL FC %	964	959	969	960	952	967	-0.18	-0.35	-0.01	**0.035**	965	961	969	956	952	961	-0.35	-0.50	-0.20	**<0.001**	**<0.001**	0.911	0.142
XS VLDL TAG %	1197	1162	1234	1195	1153	1237	-0.01	-0.15	0.14	0.912	1164	1130	1200	1226	1191	1261	0.30	0.17	0.43	**<0.001**	**0.004**	0.897	**0.002**
Large LDL																							
L LDL PL %	1322	1314	1329	1335	1327	1344	0.38	0.23	0.53	**<0.001**	1321	1314	1329	1328	1319	1337	0.21	0.07	0.36	**0.003**	**<0.001**	0.478	0.120
L LDL C %	4349	4336	4362	4321	4306	4337	-0.46	-0.59	-0.34	**<0.001**	4353	4341	4366	4328	4315	4342	-0.40	-0.52	-0.29	**<0.001**	**<0.001**	0.472	0.494
L LDL CE %	3254	3240	3268	3226	3211	3241	-0.42	-0.56	-0.29	**<0.001**	3248	3234	3263	3235	3218	3251	-0.23	-0.35	-0.10	**<0.001**	**<0.001**	0.833	**0.040**
L LDL FC %	1094	1084	1104	1094	1083	1105	0.01	-0.13	0.14	0.938	1103	1094	1113	1092	1082	1102	-0.19	-0.31	-0.06	**0.003**	0.051	0.529	**0.039**
L LDL TAG %	324	314	334	337	325	351	0.28	0.15	0.41	**<0.001**	320	310	330	338	327	350	0.33	0.21	0.45	**<0.001**	**<0.001**	0.719	0.552
Medium LDL																							
M LDL PL %	1524	1513	1536	1535	1522	1548	0.20	0.03	0.37	**0.020**	1540	1529	1552	1526	1513	1540	-0.20	-0.36	-0.05	**0.011**	0.989	0.600	**0.001**
M LDL C %	4189	4175	4203	4166	4148	4184	-0.36	-0.52	-0.20	**<0.001**	4173	4160	4186	4175	4158	4191	-0.03	-0.18	0.11	0.672	**<0.001**	0.643	**0.003**
M LDL CE %	3108	3082	3135	3083	3051	3115	-0.20	-0.36	-0.05	**0.011**	3064	3037	3091	3096	3065	3128	0.19	0.05	0.34	**0.007**	0.944	0.329	**<0.001**
M LDL FC %	1075	1056	1093	1076	1056	1098	0.02	-0.12	0.16	0.754	1103	1084	1121	1071	1052	1092	-0.30	-0.43	-0.17	**<0.001**	**0.005**	0.304	**0.001**
M LDL TAG %	282	274	290	293	283	304	0.30	0.16	0.43	**<0.001**	281	273	290	294	285	303	0.31	0.19	0.43	**<0.001**	**<0.001**	0.959	0.877

Table S3. Continued

	LFHP										HMUFA										Between-group P		
	week 0			week 12			Standardised mean change				week 0			week 12			Standardised mean change						
	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Mean	95% CI		Mean	95% CI		Mean	95% CI		P	Time	Group	Group x Time
Small LDL																							
S LDL PL %	1864	1849	1879	1893	1875	1911	0.37	0.22	0.52	**<0.001**	1882	1869	1894	1875	1860	1890	-0.08	-0.22	0.06	0.242	**0.007**	0.851	**<0.001**
S LDL C %	3798	3785	3812	3762	3742	3782	-0.50	-0.66	-0.34	**<0.001**	3791	3780	3802	3780	3766	3793	-0.20	-0.35	-0.06	**0.006**	**<0.001**	0.708	**0.007**
S LDL CE %	2775	2756	2794	2743	2720	2767	-0.32	-0.48	-0.17	**<0.001**	2752	2736	2769	2763	2744	2783	0.11	-0.03	0.26	0.125	0.055	0.821	**<0.001**
S LDL FC %	1019	1004	1034	1015	997	1032	-0.06	-0.21	0.09	0.451	1035	1020	1050	1012	996	1029	-0.31	-0.45	-0.18	**<0.001**	**<0.001**	0.540	**0.013**
S LDL TAG %	332	321	342	338	325	351	0.13	-0.01	0.28	0.072	320	309	332	338	326	351	0.35	0.22	0.48	**<0.001**	**<0.001**	0.472	**0.031**
Very large HDL																							
XL HDL PL %	2399	2224	2589	2621	2555	2689	0.19	0.12	0.27	**<0.001**	2527	2431	2626	2565	2482	2650	0.06	-0.02	0.13	0.136	**<0.001**	0.429	**0.012**
XL HDL C %	3105	3062	3150	3032	2989	3075	-0.30	-0.41	-0.20	**<0.001**	3080	3028	3133	3054	3004	3104	-0.16	-0.26	-0.07	**0.001**	**<0.001**	0.769	0.060
XL HDL CE %	2096	2060	2132	2053	2025	2082	-0.20	-0.30	-0.10	**<0.001**	2112	2085	2140	2088	2060	2115	-0.13	-0.23	-0.04	**0.007**	**<0.001**	0.214	0.355
XL HDL FC %	989	951	1029	967	932	1004	-0.08	-0.17	0.00	**0.040**	953	915	992	956	921	992	-0.02	-0.09	0.06	0.686	0.074	0.193	0.214
XL HDL TAG %	349	320	381	304	280	330	-0.28	-0.40	-0.16	**<0.001**	306	282	333	317	292	344	0.05	-0.06	0.16	0.395	**0.005**	0.325	**<0.001**
Large HDL																							
L HDL PL %	3053	2935	3176	3122	3089	3155	0.16	-0.04	0.36	0.121	3099	3064	3135	3104	3073	3136	0.01	-0.18	0.20	0.924	0.236	0.705	0.292
L HDL C %	2340	2141	2558	2488	2401	2579	0.11	0.00	0.22	**0.045**	2495	2410	2582	2512	2451	2573	0.01	-0.09	0.12	0.802	0.104	0.216	0.200
L HDL CE %	1695	1490	1928	1859	1782	1940	0.15	0.01	0.29	**0.041**	1853	1770	1939	1886	1831	1943	0.03	-0.10	0.16	0.666	0.076	0.193	0.236
L HDL FC %	596	550	647	622	601	644	0.10	0.00	0.20	0.057	632	619	644	622	611	633	-0.03	-0.13	0.07	0.517	0.350	0.346	0.068
L HDL TAG %	369	333	408	327	299	359	-0.24	-0.37	-0.10	**0.001**	325	297	355	336	310	365	0.06	-0.06	0.19	0.321	0.070	0.383	**0.002**
Medium HDL																							
M HDL PL %	2907	2891	2923	2918	2903	2933	0.15	0.02	0.27	**0.024**	2889	2875	2903	2898	2884	2912	0.10	-0.02	0.22	0.089	**0.005**	**0.023**	0.609
M HDL C %	2708	2674	2742	2711	2680	2742	0.02	-0.12	0.15	0.821	2748	2717	2780	2732	2702	2762	-0.08	-0.21	0.04	0.206	0.487	0.106	0.304
M HDL CE %	2205	2174	2237	2219	2192	2247	0.09	-0.05	0.24	0.219	2239	2211	2268	2236	2210	2263	0.00	-0.14	0.13	0.944	0.393	0.132	0.342
M HDL FC %	501	494	509	490	482	499	-0.28	-0.38	-0.18	**<0.001**	508	501	515	495	487	502	-0.35	-0.44	-0.26	**<0.001**	**<0.001**	0.268	0.327
M HDL TAG %	370	354	386	359	342	377	-0.11	-0.27	0.04	0.139	345	328	363	357	340	374	0.13	-0.01	0.27	0.074	0.896	0.196	**0.022**
Small HDL																							
S HDL PL %	3404	3394	3415	3420	3407	3433	0.30	0.17	0.43	**<0.001**	3412	3399	3424	3416	3404	3428	0.18	0.06	0.30	**0.003**	**<0.001**	0.593	0.172
S HDL C %	2279	2264	2293	2269	2252	2285	-0.16	-0.33	0.01	0.065	2286	2270	2302	2273	2257	2288	-0.21	-0.37	-0.06	**0.007**	**0.002**	0.617	0.624
S HDL CE %	1679	1666	1693	1678	1662	1694	-0.05	-0.21	0.12	0.561	1682	1666	1698	1679	1664	1695	-0.08	-0.23	0.08	0.325	0.274	0.852	0.809
S HDL FC %	598	593	604	590	584	596	-0.31	-0.42	-0.19	**<0.001**	602	597	608	593	588	598	-0.39	-0.50	-0.29	**<0.001**	**<0.001**	0.444	0.266
S HDL TAG %	311	301	322	306	295	318	-0.08	-0.22	0.06	0.286	294	282	306	305	294	316	0.17	0.04	0.30	**0.011**	0.343	0.174	**0.012**

Values are geometric means with 95% confidence intervals. The differences in metabolite area under the curves (AUCs) between the diets were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates.

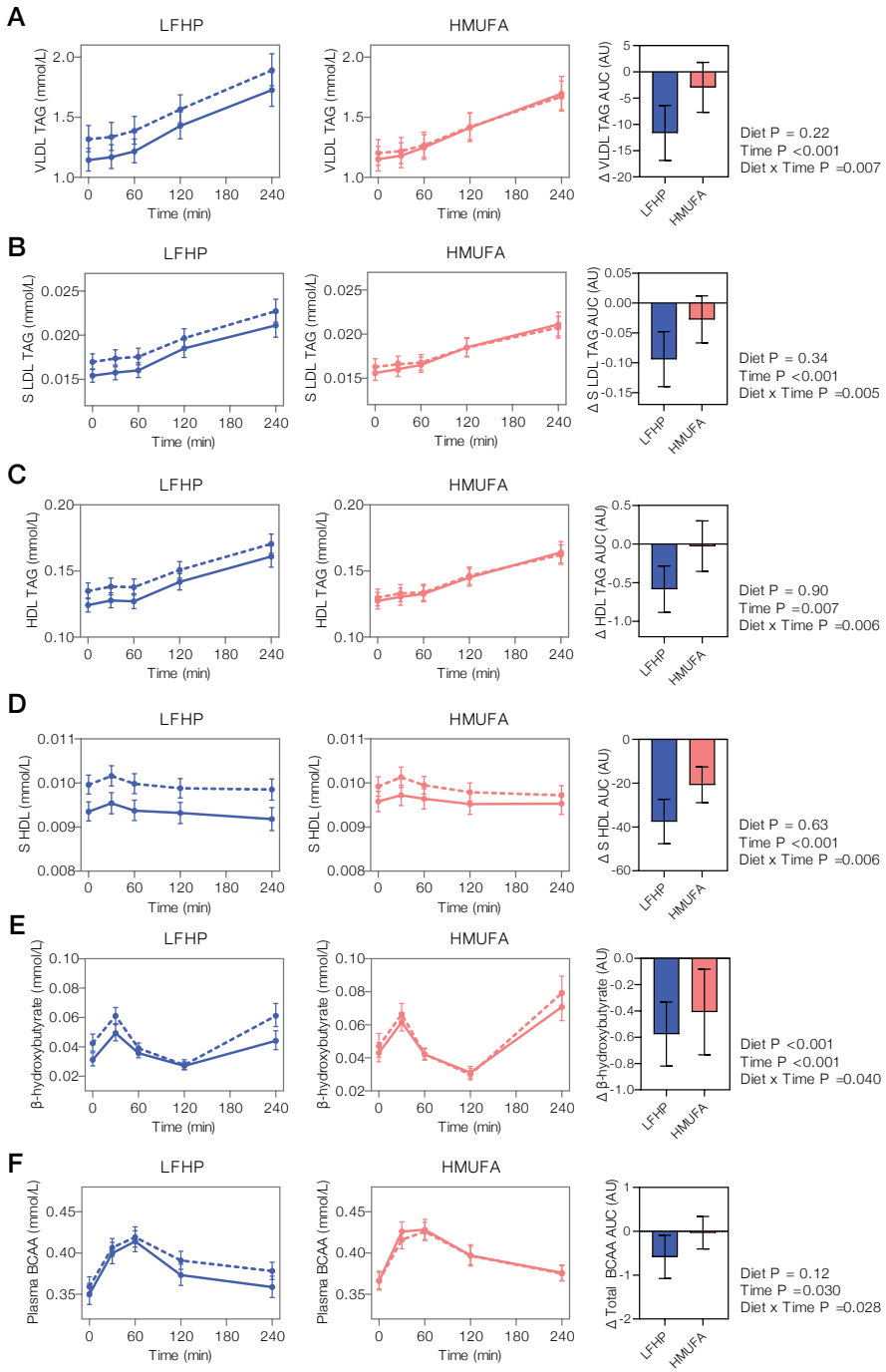

Figure S1. Plasma concentrations of VLDL-TAG (A), S LDL TAG (B), HDL-TAG (C), S HDL particles (D), β-hydroxybutyrate (E), and BCAA (F) in response to the high-fat mixed meal at week 0 (dotted lines) and week 12 (solid lines) upon the LFHP and HMUFA diet. Data are presented as geometric means with 95% CI. The differences in metabolite area under the curves (AUCs) between the diets were assessed using a linear mixed model with repeated measures and age, sex, and centre as covariates.

8

General discussion

A growing proportion of worldwide morbidity and mortality is attributable to poor diet.[1-4] Suboptimal diet quality increases the risk of cardiometabolic diseases by promoting intermediate risk factors such as high blood pressure and elevated blood glucose, triglyceride, and cholesterol levels, or can impair metabolic health via the development of adiposity, which is upstream of most cardiometabolic diseases. Improving diet quality is, therefore, a great strategy for the prevention of cardiometabolic diseases. However, the mechanisms that underlie diet-induced beneficial effects are often poorly understood. Besides, there is great inter-individual variation in how people respond to foods, meals, or diet, and emerging evidence indicates that metabolic heterogeneity may partly account for that variation. A better characterisation of metabolic heterogeneity and a better understanding of the mechanisms by which dietary modification affects metabolic health may provide leads for more successful dietary interventions that better target these metabolic impairments. The research in this thesis aimed to contribute to a better understanding of the role of metabolic heterogeneity in response to diet, with a specific focus on tissue-specific insulin resistance. In addition, we aimed to contribute to a better understanding of the mechanisms by which diet affects metabolic health by investigating circulating metabolites that are related to cardiometabolic health and liver health or function.

Summary of main findings

FGF21 is a hepatokine that is involved in energy and nutrient metabolism. Its metabolic regulation and physiological functions are highly complex and poorly understood, but circulating FGF21 levels have been proposed to be a marker of metabolic health status.[5] Our group previously demonstrated that a weight loss diet with 25% energy restriction and high nutrient quality resulted in greater weight loss and a more anti-atherogenic blood lipid profile than a diet with similar energy restriction but low nutrient quality, while both diets reduced insulin resistance and liver fat to a similar extent.[6] In **Chapter 2**, we aimed to explore whether circulating FGF21 levels are a marker of metabolic health by investigating whether these health improvements were reflected by changes in circulating FGF21 levels. We found that a high- or low-nutrient-quality weight-loss diet did not affect fasting or postprandial plasma FGF21 levels, nor did overall energy restriction. We concluded that changes in plasma FGF21 are not a sensitive marker of diet-induced changes in metabolic health in relatively healthy overweight individuals.

Fatty liver is an increasingly prevalent condition that is closely associated with obesity and insulin resistance. Our group previously demonstrated that a 12-week refined wheat intervention modestly increased liver fat (+1.5% percentage points), while whole-grain wheat did not affect liver fat content in overweight individuals with

mildly elevated cholesterol levels.[7] In **Chapter 3**, we aimed to explore potential underlying mechanisms of the preventive effect of whole-grain wheat consumption or the detrimental effect of refined wheat consumption on liver fat accumulation. To that end, we investigated the effects of these wheat interventions on plasma metabolites involved in lipid metabolism, including betaine, choline, acylcarnitines, bile acids, and signalling lipids. We found that the wheat interventions did not robustly affect these circulating metabolites. These findings suggest that the effects of the wheat interventions on liver fat accumulation were likely not mediated by the increased intake of betaine and choline from whole-wheat grain or by effects of the wheat intervention on bile acid metabolism.

The pathophysiology of whole-body insulin resistance is characterised by great heterogeneity, with inter-individual differences in the development of insulin resistance in the various metabolic organs, including the liver and skeletal muscle. This metabolic heterogeneity may be a target for precision nutrition strategies. For the first time, we prospectively studied in a randomised trial whether individuals with tissue-specific insulin resistance benefit from different diets (the PERSON study). The design, rationale, and preliminary screening results of the PERSON study are described in **Chapter 4**. In **Chapter 6**, we demonstrated that individuals with predominant muscle insulin resistance (muscle IR) benefitted more from a 12-week low-fat, high-protein, and high-fibre (LFHP) diet. In contrast, individuals with predominant liver insulin resistance (liver IR) had greater improvements upon a high-MUFA (HMUFA) diet with respect to peripheral insulin sensitivity, glucose tolerance, fasting serum TAG, and CRP. No effects were observed on the primary outcome disposition index. These findings indicate that precision nutrition based on metabolic phenotype may be superior to a one-size-fits-all diet based on general dietary guidelines for improving cardiometabolic health.

In **Chapter 7**, we further characterised the effects of the LFHP and HMUFA diets on fasting and postprandial plasma metabolite profile to identify leads towards potential underlying mechanisms of the differential effects of HMUFA and LFHP diets in tissue-specific IR. We demonstrated that the greater reduction in fasting TAG in the phenotype-diet combinations observed in **Chapter 6** was due to a larger reduction of TAG in hepatically derived VLDL particles, and not due to changes in TAG in the other lipoproteins. Furthermore, we showed that overall, irrespective of IR phenotype, the LFHP diet resulted in larger reductions in fasting and postprandial plasma concentrations of small HDL particles and almost all subclasses of VLDL particles, TAG fractions in VLDL, small LDL, and HDL, β-hydroxybutyrate, and postprandial BCAA concentrations as compared to the HMUFA diet. We concluded that the observed phenotype-diet interactions in **Chapter 6** might be related to diet-induced effects on the delivery of lipids to the liver and/or hepatic lipid storage or secretion. Nevertheless, a diet low in fat and rich in protein and fibre may be more effective for improving postprandial lipid and BCAA metabolism than a diet rich in MUFA for both individuals with liver or muscle IR.

In **Chapter 5**, we aimed to better characterise metabolic heterogeneity with respect to fasting and postprandial metabolism in tissue-specific insulin resistance. To that end, we compared the fasting and postprandial plasma metabolome in response to a high-fat mixed meal between individuals with muscle IR or liver IR in a cross-sectional analysis of the PERSON study. We demonstrated that liver IR was characterised by a more dyslipidemic postprandial metabolite profile compared to muscle IR, with larger increases in postprandial large VLDL particles and triglycerides in several VLDL, LDL, and HDL subclasses in individuals with liver IR after consumption of a high-fat mixed meal. This work points towards more pronounced impairments in postprandial lipid metabolism in liver compared to muscle IR.

Phenotype-diet interactions in cardiometabolic health improvements

With the PERSON study, we provided proof that precision nutrition based on IR phenotype is effective for improving cardiometabolic health for the first time. Importantly, these effects were independent of weight loss, since both diets resulted in comparable, minor weight loss. The effects of the LFHP and HMUFA diets on insulin sensitivity, glucose homeostasis, body composition, plasma metabolites, and other cardiometabolic parameters in both IR phenotypes are summarised in Tables 1 and 2 (**Chapters 6** and **7**).

We observed clear phenotype-diet interactions in mainly the glycemic measures and whole-body or peripheral insulin sensitivity indices: in individuals with muscle IR following the LFHP diet and in individuals with liver IR following the HMUFA diet, fasting glucose, 1-hour glucose after OGTT, the Matsuda index, and the muscle insulin sensitivity index (MISI) were improved to a larger extent than in the other phenotype-diet combinations (**Chapter 6**). We speculated that the HMUFA-induced improvement in peripheral insulin sensitivity in individuals with liver IR was possibly mediated partly via improved hepatic lipid metabolism, also because of the observed HMUFA-induced reduction in plasma total TAG in liver, but not muscle IR. Using more extensive metabolomics analyses, we demonstrated in **Chapter 7** that the HMUFA diet specifically lowered the fasting VLDL-TAG fraction in liver IR, but did not affect postprandial VLDL-TAG. Interestingly, the LFHP diet resulted in greater changes in postprandial plasma VLDL and HDL profiles indicative of improved hepatic lipid metabolism than the HMUFA diet in both IR phenotypes. Therefore, the HMUFA-induced improvements in peripheral insulin sensitivity in liver IR observed in **Chapter 6** are likely not only mediated by improved hepatic lipid metabolism. Potential other mediators remain to be elucidated.

The LFHP diet induced greater improvements in glycemic markers and peripheral insulin sensitivity in individuals with muscle IR than in individuals with liver IR (**chapter 6**). We speculated that the observed beneficial effects of the LFHP diet on peripheral insulin sensitivity in muscle IR might have been mediated via modulation of the gut microbiota by the high-fibre consumption in the LFHP diet. We indeed recently observed that individuals with muscle IR had a less favourable gut microbial profile compared to those with liver IR, which indicates that a dietary intervention rich in fibre that specifically targets the gut may induce larger benefits in muscle IR (Jardon et al., in preparation). The effects of the diets and phenotype-diet interactions on gut microbiome composition and related gut health parameters are currently under investigation. Furthermore, the LFHP-induced improvements in peripheral insulin sensitivity in individuals with muscle IR may be mediated by effects on inflammatory parameters. High fibre consumption has previously been shown to reduce CRP.[8] We (Trouwborst et al., in print) and others[9] have previously shown that muscle IR is associated with increased systemic inflammation and a more inflammatory gene expression profile in SAT. Here, we also observed that individuals with muscle IR had elevated C-reactive protein (CRP) levels compared to liver IR at baseline (**Chapter 5**). The LFHP diet reduced CRP levels in muscle IR, indicative of a reduction in systemic inflammation (**Chapter 6**). This reduction in systemic inflammation may have been accompanied by amelioration of adipose tissue inflammation, which consequently may have resulted in reduced peripheral insulin resistance. Investigation of LFHP-induced effects on SAT inflammation is warranted by analysis of inflammatory gene and protein expression and immune cell composition in the abdominal SAT (aSAT) biopsies collected in the PERSON study. In addition, diet-induced effects on systemic inflammation can be further explored by analysis of gene expression and immune cell composition of the collected peripheral blood mononuclear cells (PBMCs).

Diet-specific effects on cardiometabolic health outcomes

Both the LFHP and the HMUFA diet resulted in meaningful health benefits in both IR phenotypes, despite only minor weight loss of ~2%. Apart from the earlier-mentioned reduction in liver fat, both diets lowered plasma fasting and postprandial concentrations of LDL and IDL particles (**Chapter 7**). These particles carry about 50% of all plasma cholesterol,[10] and hence, both diets lowered plasma cholesterol. In addition, both diets lowered fasting and postprandial plasma SFA fraction (**Chapter 7**). Although these effects may have also been partly related to beneficial effects of the high MUFA intake on the HMUFA diet, and the lower fat and higher protein and fibre consumption on the LFHP diet, they likely are largely due to the relatively low SFA intake on both diets of ~8.5 energy%.[11,12] Interestingly, lower fasting plasma SFA fraction, total cholesterol, LDL cholesterol, IDL cholesterol, and LDL and IDL particle concentrations

Table 1. Effects of the 12-week LFHP and HMUFA diets in individuals with muscle IR or liver IR on markers of glucose metabolism, cardiometabolic markers, body composition, and ectopic fat

	Low-fat, high-fibre, high-protein diet		High-MUFA diet	
	Muscle IR	Liver IR	Muscle IR	Liver IR
Glucose metabolism				
Fasting glucose$_{OGTT}$	↓	↔	↓	↓
1-hour glucose$_{OGTT}$	↓	↔	↔	↓
2-hour glucose$_{OGTT}$	↓	↔	↔	↔
Glucose iAUC$_{OGTT}$	↓	↔	↔	↓
Glucose iAUC$_{HFMM}$	↓	↔	↓↓	↓↓
Fasting insulin$_{OGTT}$	↓	↔	↔	↓
2-hour insulin$_{OGTT}$	↓	↔	↔	↓
Insulin iAUC$_{OGTT}$	↓	↓	↔	↔
Insulin iAUC$_{HFMM}$	↔	↓	↔	↔
HOMA-IR	↓	↔	↔	↓
Matsuda index	↑↑	↑	↔	↑↑
Insulinogenic index[a]	↓	↓	↔	↔
Disposition index	↔	↔	↔	↔
HIRI	↓↓	↓↓	↔	↓
MISI	↑	↔	↔	↑
HbA1c	↔	↔	↔	↔
Other cardiometabolic markers				
Adipose tissue IR	↓	↔	↓	↓
Fasting plasma NEFA	↓	↔	↓↓	↔
Systolic blood pressure	↓	↓	↔	↓
Diastolic blood pressure	↓	↓	↔	↔
C-reactive protein	↓	↔	↔	↔
Body composition and ectopic fat				
Body fat %	↓	↓	↓	↓
Android fat	↓	↓	↓	↓
Gynoid fat	↓	↓	↓	↓
VAT	↓↓	↓↓	↓↓	↓
Liver fat	↓	↓	↓	↓
Muscle fat infiltration	↔	↓	↔	↔

[a] Calculated as insulin AUC$_{0\text{-}30}$ / glucose AUC$_{0\text{-}30}$

Arrows indicate a significant within-group change (↓↑) or no within-group change (↔). Double arrows indicate larger effect size compared to a single arrow between phenotype-diet groups.

Table 2. Effects of the 12-week LFHP and HMUFA diets in individuals with muscle IR or liver IR on fasting and postprandial (AUC) plasma metabolites in response to a high-fat mixed meal

	Low-fat, high-fibre, high-protein diet		High-MUFA diet	
	Muscle IR	Liver IR	Muscle IR	Liver IR
Lipids and lipoproteins				
Total cholesterol				
Fasting	↓	↓	↓	↓
Postprandial	↓	↓	↓	↓
LDL cholesterol				
Fasting	↓	↓	↓	↓
Postprandial	↓	↓	↓	↓
HDL cholesterol				
Fasting	↓	↔	↓	↔
Postprandial	↓	↔	↓	↔
Remnant cholesterol				
Fasting	↓	↓	↓	↓
Postprandial	↓	↓	↓	↓
Total TAG				
Fasting	↓	↓	↔	↓
Postprandial	↓	↓	↔	↔
VLDL TAG				
Fasting	↓	↓	↔	↓
Postprandial	↓	↓	↔	↔
LDL TAG				
Fasting	↓	↓	↓	↓
Postprandial	↓↓	↓↓	↓	↓↓
HDL TAG				
Fasting	↓	↓	↔	↔
Postprandial	↓	↓	↔	↔
VLDL size				
Fasting	↓	↔	↔	↔
Postprandial	↔	↔	↑	↔
LDL size				
Fasting	↔	↔	↓	↔
Postprandial	↔	↑	↓	↔

Table 2. Continued

	Low-fat, high-fibre, high-protein diet		High-MUFA diet	
	Muscle IR	Liver IR	Muscle IR	Liver IR
HDL size				
Fasting	↔	↔	↔	↔
Postprandial	↑	↑	↔	↔
Other metabolites				
Branched-chain amino acids				
Fasting	↔	↔	↔	↔
Postprandial	↓	↔	↔	↔
β-hydroxybutyrate				
Fasting	↓	↓	↔	↔
Postprandial	↓↓	↓	↓	↔

Arrows indicate a significant within-group change (↓↑) or no within-group change (↔). Double arrows indicate larger effect size compared to a single arrow between phenotype-diet groups.

are associated with reduced risk of future CVD[13], but not T2DM.[14] These findings illustrate the great potential of improving diet quality for reducing cardiovascular risk, without substantial weight loss and irrespective of IR phenotype. Importantly, we did not include a control group, so additional factors other than the dietary intervention may have also contributed to the observed effects.

Nevertheless, we also observed diet-specific effects. Irrespective of IR phenotype, the LFHP diet resulted in greater changes in plasma metabolite profiles compared to the HMUFA diet (**Chapter 7**), many of which are indicative of reduced future risk of CVD and/or T2DM[13-18]. A potential explanation for the LFHP-induced effects on plasma VLDL profile may be reduced VLDL production due to lower hepatic substrate availability. The observed reductions in fasting and postprandial concentrations of the ketone body β-hydroxybutyrate upon the LFHP diet point towards lower NEFA supply from (visceral) adipose tissue lipolysis to the liver.[19] VLDL production is largely determined by substrate availability,[20,21] and hence, lower NEFA supply may have resulted in decreased VLDL production, thereby reducing postprandial VLDL-TAG. We also observed a greater improvement in hepatic insulin sensitivity upon the LFHP diet compared to the HMUFA diet (**Chapter 7**), which may have contributed to reduced postprandial VLDL-TAG by better insulin-mediated suppression of VLDL production.[22-24] The potentially lower NEFA delivery to the liver may have also contributed indirectly to the observed improvement in hepatic insulin sensitivity by

reducing hepatic substrate availability for gluconeogenesis, thereby reducing hepatic glucose production.[25] However, these processes are closely related, and the order in which these processes took place cannot be deducted from our study. Taken together, our findings indicate that a diet low in fat and rich in fibre and protein is more effective at improving hepatic lipid metabolism than a diet rich in MUFA for both IR phenotypes, potentially via increasing hepatic insulin sensitivity and reducing NEFA supply from adipose tissue lipolysis. Analysis of mRNA expression of genes involved in insulin signalling, lipolysis, and adipogenesis in collected aSAT biopsies is warranted to explore diet-induced effects on adipose tissue further.

Furthermore, the LFHP diet reduced postprandial concentrations of BCAA and glutamine (**Chapter 7**), an intermediary metabolite of BCAA metabolism, compared to the HMUFA diet. Interestingly, this effect appeared to be more prominent in individuals with muscle IR compared to those with liver IR, despite similar fasting and postprandial plasma BCAA at baseline (**Chapter 5**). This indicates that the LFHP-induced effects on postprandial BCAA may be specific for individuals with muscle IR. The observed reduction in postprandial plasma BCAA appeared to be mainly accounted for by a steeper decrease after the peak one hour after consumption of the mixed meal, suggesting increased BCAA degradation. Dysfunctional BCAA catabolism in skeletal muscle, adipose tissue, and liver has been shown to be closely related to insulin resistance in animal studies, but the temporal and causal relationship is unclear.[26] A recent trial[27] demonstrated that pharmacologically enhancing BCAA catabolism increased peripheral insulin sensitivity in patients with T2DM, mainly by enhancing skeletal muscle glucose oxidation, and with limited effects on hepatic insulin sensitivity. Hence, the observed effects on plasma BCAA may be related to the LFHP-induced improvement in insulin sensitivity. However, we cannot investigate in our study whether the reduced postprandial BCAA would be a cause or consequence of increased insulin sensitivity. Potential LFHP-induced effects on BCAA catabolism can be further explored by analysing gene and protein expression of BCAA catabolic enzymes in collected skeletal muscle and sAT biopsies.

The MUFA in the HMUFA diet came mainly from olive oil, which is one of the key components of the Mediterranean diet. Ever since Ancel Keys proposed in the 1960s that the very low rates of CVD in Southern Europe may be due to their Mediterranean dietary patterns,[28] health effects of these diets have been intensively studied. The Mediterranean diet is now considered one of the most beneficial dietary patterns for cardiometabolic health.[29] In two previous large randomised controlled trials - the PREDIMED trial and the CORDIOPREV study - the MUFA-rich Mediterranean diet outperformed a control or low-fat diet in improving blood lipid profile and reducing incident CVD and T2DM.[29-34] We found that the HMUFA diet reduced plasma cholesterol to a similar extent as the LFHP diet, but – unlike the LFHP diet - did not affect postprandial VLDL-TAG and HDL-TAG (**Chapter 7**). These findings indicate that a LFHP diet may confer additional cardiometabolic health benefits over a HMUFA diet in individuals with tissue-specific IR. The PREDIMED trial and CORDIOPREV study differed in various aspects from our study, including a study population with CVD or increased risk of CVD, overrepresentation of individuals with T2DM, and less intensive dietary counselling on the control or low-fat diet compared to the Mediterranean diet. Evidently, the contrasting results may also be due to differences in the macronutrient composition of the control or low-fat diet used in PREDIMED and CORDIOPREV, as compared to the LFHP in our study, including higher fat and lower protein and fibre content. Nevertheless, the observed limited effects of the HMUFA diet on postprandial blood lipids may also indicate that components of the Mediterranean diet other than the high MUFA content, such as dietary fibre or PUFA-rich foods including nuts and fatty fish, may confer additional health benefits, which is indeed supported by some studies.[35-37] Thus, while MUFA consumption may induce various health benefits, the most optimal health effects are likely conferred by a complete Mediterranean dietary pattern, which was not tested in our study.

We found that individuals with liver IR had a more dyslipidemic postprandial metabolite profile on average as compared to those with muscle IR, characterised by larger increases in postprandial large VLDL particles and triglycerides in several VLDL, LDL, and HDL subclasses after consumption of a high-fat mixed meal (**Chapter 5**). Nevertheless, the variation between individuals with liver IR was considerable. Hence, normolipidemic individuals with liver IR might benefit more from a HMUFA diet resulting in improved systemic insulin resistance, while individuals with liver IR and dyslipidemia may benefit more from a LFHP diet by improving hepatic lipid metabolism. This finding illustrates that more extensive metabolic phenotyping - not only phenotyping according to tissue-specific insulin resistance, but also on lipemic measures - may be required to further refine personalised dietary advice.

Inter-individual variation in cardiometabolic health improvements

Although precision nutrition is founded upon the concept of inter-individual variability in dietary response, many precision nutrition approaches are performed at the subgroup level rather than at the individual level, as was our study. On average, individuals with muscle IR benefitted more from the LFHP diet with respect to whole-body insulin sensitivity, whereas individuals with liver IR benefitted more from the HMUFA diet on average (**Chapter 6**). However, as expected, individual responses to the diets varied quite considerably within the IR phenotypes. Individual changes in the Matsuda index upon the dietary intervention are depicted according to IR phenotype in Figure 1. The observed variation in phenotype-diet effects might partly be explained by inter-individual differences in dietary adherence, but other factors, including habitual diet, genetics, baseline health status, physical activity, stress, and sleep, may also be involved. This variation clearly illustrates that there is still room for

improvement in phenotyping. The extensive data we collected in the PERSON study provides a great source for investigation and possible identification of which additional factors affect response to diet. Precision nutrition strategies may be optimised by further refining metabolic phenotypes based on these parameters.

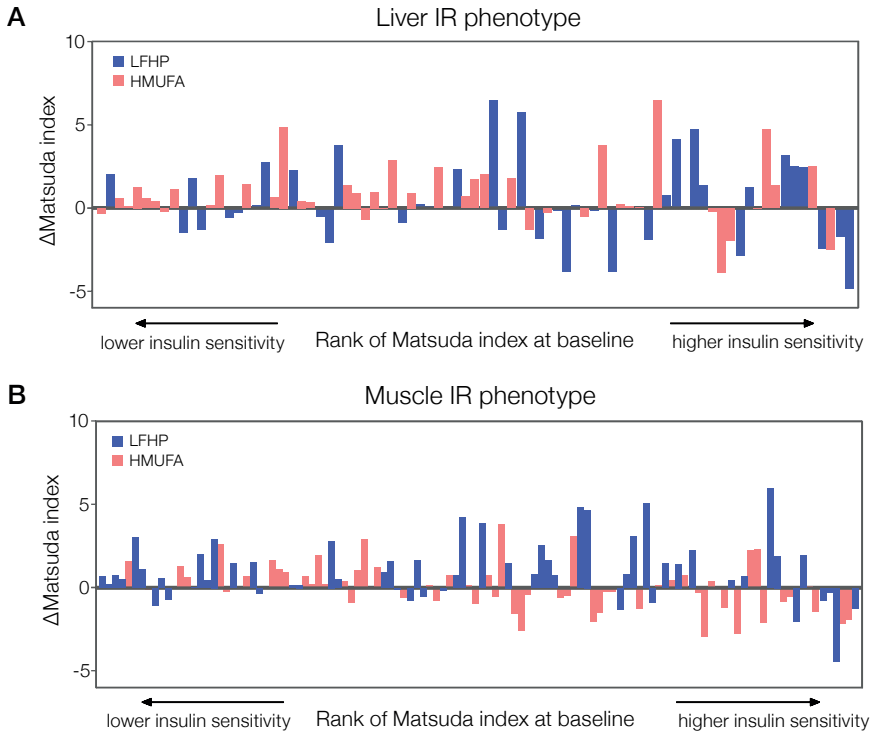

Figure 1. Individual changes in the Matsuda index upon the 12-week LFHP (blue) or HMUFA (red) diet in individuals with muscle insulin resistance (A) or liver insulin resistance (B) ranked according to baseline Matsuda index from low (left) to high (right). Higher Matsuda index indicates better insulin sensitivity.

Sub-phenotyping insulin resistance

Metabolic heterogeneity in tissue-specific insulin resistance

We hypothesised that individuals with different forms of tissue-specific insulin resistance would respond differentially to a dietary intervention due to their metabolic differences. Previously, insulin resistance in the liver has been associated with visceral adiposity, elevated liver fat, and abnormalities in blood metabolome,[38-40] while insulin resistance in muscle has been associated with systemic and adipose tissue inflammation,[9] as well as with increased muscle fat infiltration (Trouwborst et al., in preparation). We extended these findings to the postprandial plasma metabolome, showing that individuals with liver IR had a more dyslipidemic postprandial plasma profile in response to a high-fat mixed meal than individuals with muscle IR, indicative of more impaired postprandial lipid metabolism (**Chapter 5**). These results confirm previously reported elevated postprandial triglycerides in liver compared to muscle IR.[40] The higher postprandial plasma VLDL and SFA fraction observed in individuals with liver compared to muscle IR may point to higher *de novo* lipogenesis (DNL). Higher hepatic IR, as measured with the gold-standard hyperinsulinemic-euglycemic clamp method, has indeed been associated with elevated DNL.[41] Whether postprandial dyslipidemia is a cause, consequence, or simply a concurrent feature of hepatic insulin resistance has yet to be elucidated and perhaps also differs between individuals.[42]

Aetiology of tissue-specific insulin resistance

Why the rate and severity of development of tissue-specific insulin resistance differ between individuals is poorly understood and likely involves complex interactions between genetic, epigenetic, environmental, and behavioural factors including diet and physical activity. The liver receives most of its blood from the portal vein, and is consequently exposed to relatively high concentrations of mediators deriving from the gut, spleen, and visceral adipose tissue.[43] Metabolic perturbations in these tissues can lead to increased portal delivery of pro-inflammatory cytokines or NEFA to the liver, thereby potentially promoting ectopic fat accumulation and insulin resistance in the liver. The elevated postprandial plasma VLDL we observed in individuals with liver IR compared to muscle IR (**Chapter 5**) could possibly also be explained by higher substrate availability from VAT lipolysis, since VAT is highly lipolytic[44] and more resistant to insulin suppression than SAT[45]. In contrast, skeletal muscle may be more strongly affected by the tissues that drain into the systemic circulation, including the subcutaneous adipose tissue. The latter may explain the observed elevated systemic and sAT inflammation in muscle IR by us (**Chapter 6**, Trouwborst et al., in print) and others[9]. Importantly, however, these cross-sectional studies do not allow for any conclusions on the temporal and causal relationships of these factors.

The development of tissue-specific insulin resistance also seems to be characterised by sex differences: we found women to be overrepresented in the muscle IR group, while men were overrepresented in the liver IR group (**Chapter 4**), which is in line with previous studies[39]. The observed sex-specific associations between HIRI and postprandial lipemia (**Chapter 7**) further support potential sexual dimorphism in the development of tissue-specific insulin resistance.

Regarding lifestyle factors, we recently found that increased sedentary behaviour, as assessed objectively with an activity monitor, was associated with lower muscle, but not liver, insulin sensitivity.[46] Indeed, exercise is well-known for improving skeletal muscle insulin sensitivity.[47] Habitual dietary intake did not differ between individuals with liver IR, muscle IR, or no tissue-specific insulin resistance in an interim analysis of screening data from the PERSON study in 565 individuals, at least not with respect to macronutrient composition (**Chapter 4**). However, in the final study population of included participants, we observed that individuals with liver IR had lower overall diet quality, as assessed by the Dutch Healthy Diet index[48], compared to individuals with muscle IR, an effect that was driven by differences in men only (**Chapter 5**). It would be interesting to repeat these analyses in the total screening population of 877 individuals and additionally investigate habitual dietary intake on the food group or dietary pattern level, since that gives more information about habitual diet than macronutrient intake alone.

Measuring tissue-specific insulin resistance

The gold standard method for quantifying insulin resistance - the two-step hyperinsulinemic-euglycemic clamp technique – is very burdensome, labour-intensive, and costly, and simpler surrogate measures for estimating whole-body and tissue-specific insulin resistance have therefore been developed. In **Chapters 4-7**, we used measures derived from a 7-point OGTT to assess liver and insulin resistance. The hepatic insulin resistance index (HIRI) is calculated by multiplying the areas under the curve for glucose and insulin in the first 30 minutes of the OGTT, which reflects the insulin-mediated suppression of endogenous glucose production. The muscle insulin sensitivity index is calculated by dividing the glucose decline rate from peak to nadir by the average insulin concentration, which reflects glucose uptake in the peripheral tissues in the late postprandial phase, which is accounted for mainly by skeletal muscle. These indices have been validated against clamp-derived measures, showing moderate to strong correlations of 0.5-0.8.[49,50] Glucose and insulin responses to an OGTT do not reflect pure insulin action because they are also affected by gastrointestinal factors, including gastric emptying, intestinal absorption, and incretin response when glucose is orally ingested.[51,52] As such, inter-individual differences in OGTT-derived measures may also partly result from differences in these gastrointestinal factors. Nevertheless, measures based on the oral ingestion of glucose rather than intravenous infusion may better reflect physiology, and may therefore also be relevant for inter-individual differences in dietary response.

When used for metabolic phenotyping, method reproducibility is even more important than validity. We used indices derived from a highly dynamic 7-point OGTT, and glucose and insulin responses to an OGTT are well-known to be poorly reproducible.[53-55] At screening, we classified individuals as predominant liver or muscle IR using tertile cut-offs for MISI and HIRI based on values of a selected study population of The Maastricht Study. At baseline, which was within three months after screening (median 6.8 weeks), we again performed a 7-point OGTT and determined HIRI and MISI. HIRI measured at screening was reasonably well correlated to HIRI measured at baseline (Pearson's r = 0.68, Spearman's ρ = 0.63), while MISI showed a weaker correlation between the two measurements (Pearson's r = 0.32, Spearman's ρ = 0.34) (unpublished data). Some individuals may thus have been misclassified at screening. However, it is unknown whether the discrepancy in indices assessed at screening or baseline represents an actual change in muscle insulin sensitivity or is primarily due to relatively low measurement reproducibility. This may have caused some of the observed inter-individual variation in intervention response (**Chapters 6** and **7**) and in plasma metabolome differences between the IR phenotypes in the cross-sectional study (**Chapter 5**). Nevertheless, on a group level, the IR phenotypes represent predominant muscle or liver IR. This is also evident from the observed higher fasting glucose and insulin levels in liver IR and higher 2-hour glucose and insulin levels after the OGTT in muscle IR (**Chapter 5**). ImportantlyIn addition, the IR phenotypes showed clear differential responses to the two diets (**Chapter 6**), and we (**Chapter 5**) and others[9,38-40] have shown that liver and muscle IR based on OGTT-derived measures represent distinct metabolic phenotypes in different populations. This indicates that a single OGTT can be used to identify tissue-specific IR phenotypes, at least on the group level.

Although less burdensome, labour-intensive, and expensive than the clamp method, a 7-point OGTT is still relatively invasive and time-consuming and cannot be performed at home. For large-scale studies and eventual use in practice, simpler methods for estimating tissue-specific insulin resistance should be developed. While simple measures such as fasting insulin or HOMA-IR or QUICKI index, calculated from fasting insulin and glucose, provide reasonably good indications of whole-body insulin resistance,[56] the main challenge is probably developing simple surrogate measures that can sufficiently discriminate between liver and muscle IR.

Different approaches for precision nutrition

Although we demonstrated in the PERSON study that individuals responded differentially to dietary modification depending on tissue-specific IR phenotype, our findings were contrary to our hypothesis: the phenotype-diet combinations that we expected to be suboptimal induced greater health benefits than the phenotype-diet combinations that were hypothesised to be optimal. We used a hypothesis-driven, sub-group-based approach and tailored the dietary advice to the phenotypes based on a combination of *post-hoc* sub-group findings from a large dietary intervention

trial, the CORDIOPREV-DIAB study,[57] and experimental evidence[42,58-63]. Possible explanations for the discrepancy in the results of our study to those of the CORDIO-PREV-DIAB study - including differences in the study population and diet composition - are described in **Chapter 6**. It must be noted that explorative subgroup analyses are sensitive to chance findings,[64] so they should preferably be validated in other study populations before being tested in a trial. This, however, is usually difficult due to the limited availability of data from trials with comparable dietary interventions and phenotypic information. So although the validation of phenotype-diet interactions that have been consistently identified *post-hoc* in different study populations by testing them in clinical trials is still warranted, other precision nutrition approaches are also required.

Several approaches can be considered. Firstly, existing data from completed intervention trials can also be used to predict differential dietary responses using machine learning. Initially, we also re-analysed data from completed large dietary trials with the aim of identifying relevant phenotype-diet interactions, but no solid phenotype-diet interactions were found, which was mainly due to insufficient statistical power (unpublished data). Indeed, a major issue with such approaches is that they require large volumes of data and are dependent on the availability of data from completed dietary trials with sufficiently large sample size and adequate phenotypic information, which is limited. Furthermore, such an approach is dependent on the dietary interventions that have been tested previously, and therefore limited in which phenotype-diet interactions can potentially be identified. Nevertheless, if large volumes of data are available, such methods may generate interesting new hypotheses,[65] which require subsequent testing in clinical trials.

Secondly, sub-phenotyping individuals according to more traditional biomarkers such as fasting plasma glucose or cholesterol may also improve the prediction of dietary response. An advantage of this approach is that the scientific evidence for modifying these biomarkers with diet is better established than for more novel markers or markers identified with a machine learning or other data-driven approach. An example of such a strategy is the metabotype approach used by O'Donovan and colleagues.[66,67] In this study, individuals were clustered into three metabolic phenotypes – termed metabotypes – according to their fasting glucose, triglycerides, total cholesterol, and HDL cholesterol. Based on these metabotypes, targeted dietary advice was delivered, for instance, to lower cholesterol for those in the elevated cho-lesterol-metabotype. An RCT testing the effectiveness of such personalised dietary advice based on metabotype has recently been completed, and results are expected to be published soon.[68]

While the use of traditional clinical biomarkers enables relatively easy application in the clinic, these markers are primarily intended for the detection of disease. They are therefore less suitable for picking up more subtle metabolic perturbations. Hence, including additional phenotypic information or metabolites that better reflect various metabolic processes for metabotyping may be important for successful precision nutrition strategies. Hence, a second approach may be classifying individuals not only on tissue-specific insulin resistance, but also on other individual characteristics. Compared to postprandial glycemic responses, postprandial lipid responses are even more variable between individuals, less determined by genetics, and more determined by individual, modifiable factors.[69] In addition, our findings suggest that postprandial lipids, BCAA, and β-hydroxybutyrate may be involved in phenotype-diet interactions (**Chapter 7**). Postprandial responses of these metabolites may therefore be a relevant additional factor to phenotype individuals on. Other parameters that could be considered for refinement of metabolic phenotyping because they have been suggested to contribute to inter-individual variation in response to acute meals or longer-term dietary interventions include genetic variation, body composition, and gut microbiota composition.[6,57,69-75]

An example of a study that applied precision nutrition according to a comprehensive metabolic phenotype is the PREVENTOMICS study.[76] In this study, individual information on genetics and 51 blood and urine metabolites were used to cluster individuals into one of five metabotypes that represented disturbances in physiological processes related to carbohydrate metabolism, gut microbiota, lipid metabolism, inflammation, or oxidative stress. Personalised dietary advice and meals supplemented with functional foods were provided that aimed to target those metabolic perturbations and thereby promote weight loss and metabolic health benefits. Recently, a 10-week RCT[77] showed that the personalised diet did not result in greater weight loss or cardiometabolic health improvements compared to generalised advice. Importantly, these null results do not necessarily demonstrate that more personalisation of dietary advice based on specific metabolic impairments using genetic and metabolomic data holds no benefits over general advice. An alternative explanation for the personalised diets not adequately ameliorating those metabolic impairments may be that we do not yet understand well enough how to target these metabolic perturbations by diet.

Because of the practical and financial difficulty of testing a large number of different dietary interventions in extensively phenotyped individuals in longer-term RCTs, testing individuals' responses to acute meals or short-term interventions may be a feasible alternative, especially with the current availability of wearable sensors such as continuous glucose monitors (CGM) and rapid developments in methods for metabolite quantification in dried blood spots (DBS). With respect to acute meal responses, various groups have demonstrated that postprandial glycemic responses,[78] and to a somewhat lesser accuracy also postprandial triglyceride responses,[69] can be successfully predicted by a machine-learning algorithm based on data from CGM and multiple postprandial finger-pricks for measurement of blood

triglycerides by DBS in combination with clinical measures, gut microbiome composition, and meal features. Postprandial glucose and triglyceride responses are highly relevant for cardiometabolic health, but so are other postprandial metabolites such as lipoproteins, amino acids, or inflammatory markers. Hence, more extensive metabolite profiling of acute meal responses would be desirable, but is currently difficult due to the lack of simple and at-home methods for measurement of most metabolites. Nevertheless, developments in novel technologies for less invasive or at-home measurement methods are advancing rapidly, and more extensive metabolite profiling may thus be possible in the near future. For example, a wearable sensor for continuous monitoring of not only glucose, but also ketones and lactate in interstitial fluid is expected to be released soon (Abbott Lingo). Because such data-driven approaches require large volumes of data and there is a trade-off between the possible depth of phenotyping and scale with respect to sample size, these methods are currently less suitable for thoroughly investigating the mechanisms underlying phenotype-diet interactions by, for instance, analysing tissue transcriptomes. But again, advances in novel technologies may allow for more simple, affordable, and at-home deep phenotyping methods in the future. Importantly, acute meal responses may not reflect the health effects of more chronic exposure to specific nutrients or meals. Therefore, the clinical effects of dietary advice based on meal responses require longer-term testing.

Evidently, precision nutrition research is highly complex, and the different precision nutrition approaches have advantages and disadvantages. Successful precision nutrition based on metabolic phenotype relies on a detailed characterisation of the metabolic heterogeneity in metabolic phenotypes and an adequate understanding of how to target that metabolic heterogeneity by diet. Combining predictive approaches based on (existing) data of meal responses or dietary interventions with existing knowledge on metabolic heterogeneity, metabolism, diet and their interaction can be used to identify metabolic phenotypes and predict dietary response. Subsequent longer-term clinical trials are required for validation and investigation of mechanisms underlying phenotype-diet interactions.

Precision nutrition may increase motivation and adherence

Even when the efficacy of precision nutrition is firmly established, i.e. when we have elucidated the most important biological factors that affect an individual's dietary response and we can design a diet that is perfectly suited to one's biology, a diet is only effective when followed and maintained in daily life. In addition, a common critical remark about precision nutrition is that increasing overall adherence to the current dietary guidelines would be more effective for achieving health benefits. But while diet quality has improved overall in the Netherlands in the past decade (Dutch National Food Consumption Survey 2019-2021), most people still do not adhere to the national dietary guidelines. In that respect, precision nutrition may help to improve diet quality, because more personalised dietary advice may increase the motivation for and adherence to dietary modification. A recent meta-analysis of 11 RCTs, including the large European Food4Me trial, concluded that precision nutrition advice based on habitual diet, phenotype, genotype, or a combination of these factors improved dietary intake more than general nutritional advice in a healthy adult population, although effects of the individual trials were mixed.[79] Importantly, individuals that are aware of their own suboptimal metabolic health may be particularly motivated to change their behaviour to prevent future chronic disease.[80] Hence, the added value of precision nutrition may be both increasing the health effects of a dietary intervention, as well as promoting adherence to dietary advice.

Measuring circulating metabolites for a better understanding of metabolic heterogeneity and diet-induced effects

In the work described in this thesis, we quantified circulating metabolites to gain more insights into the effects of various dietary interventions and the metabolic heterogeneity in tissue-specific IR. Traditional clinical biomarkers are primarily intended for the detection of disease. They are therefore less suitable for picking up more subtle metabolic perturbations or for subtle changes in response to a dietary intervention. Moreover, these traditional biomarkers do not reflect perturbations in other metabolic pathways that are also highly relevant for the development of cardiometabolic disease, such as inflammation, oxidative stress, and insulin resistance.

In **Chapters 2** and **3**, we performed *post-hoc* secondary analyses of dietary intervention trials that benefitted metabolic health, specifically liver health, to identify leads towards potential mechanisms that underlie the observed effects. To that end, we measured the hepatokine plasma FGF21 in response to two energy restriction diets with different nutrient quality (**Chapter 2**) and plasma betaine, choline, bile acids, acylcarnitines, and signalling lipids in response to a whole-grain or refined wheat intervention (**Chapter 3**). In both studies, the circulating metabolites were not robustly affected by the dietary interventions, indicating that they were likely not involved in the diet-induced changes in metabolic health. In **Chapter 7**, we extensively characterised the effects of the LFHP and HMUFA diets on fasting and postprandial plasma metabolite profiles, as discussed above. Finally, in **Chapter 5**, we compared fasting and postprandial metabolite profiles between individuals with liver or muscle IR.

Circulating FGF21 in diet-induced weight loss and metabolic health improvements

Hepatic FGF21 production is induced by a variety of metabolic and cellular stressors.[81,82] The lack of change in plasma FGF21 we observed in **Chapter 2** upon weight loss and accompanying health improvements may possibly be related to the multitude of different stressors that affect FGF21; weight loss-induced reductions in plasma FGF21 levels may have been masked by a concurrent increase of hepatic FGF21 secretion due to the acute metabolic stress caused by the caloric restriction, since a state of caloric deficit is not a stable homeostatic state, but one of metabolic stress. In addition, circulating FGF21 levels and FGF21 responses to meals or diet are highly variable between individuals,[83] something we also clearly observed, which may reflect the complex regulation of FGF21.[5] We did observe weak correlations between the baseline postprandial FGF21 response to a mixed meal and insulin resistance, which suggests that FGF21 dynamics to an acute stimulus may be reflective of metabolic health. In conclusion, circulating FGF21 in response to an acute stimulus such as a mixed meal may be a better marker of metabolic health than plasma FGF21 in response to a complex, long-term stimulus such as a 12-week moderately energy-restricted diet. Further research on the biological interpretation of inter-individual differences in plasma FGF21 (dynamics) is warranted.

Metabolomics

The circulating metabolome results from complex interactions between genotype, lifestyle, gut microbiota, and environmental exposures, and as such, reflects a variety of biological processes. Therefore, metabolomics is frequently suggested as the omics discipline that best reflects the phenotype and provides the closest representation of health status.[84-87] At the same time, the circulating metabolome is a snap-shot read-out of all those complex biological processes and interactions. It provides no information on metabolic fluxes and intermediary metabolism in tissues and organs.[88] For instance, the lack of effect we found of whole-grain wheat and refined wheat intervention on plasma betaine, choline, and bile acids (**Chapter 3**) does not entirely exclude an involvement of these metabolites in the previously observed wheat intervention effects on liver fat, because these metabolites may, for instance, have been affected in the liver, portal vein, or small intestine. Measuring these metabolites in these biological samples would likely have provided more insights into their possible involvement because they are closer to where they potentially would have affected liver fat accumulation, but collection of liver tissue, portal blood, or intestinal content in (healthy) humans is commonly not an option for ethical, financial, and practical reasons.

In addition, the biological interpretation of changes in circulating metabolites is often difficult.[88] Some plasma metabolites reflect specific biological processes reasonably well, for instance, β-hydroxybutyrate as a marker for hepatic fatty acid oxidation, which we measured in **Chapters 5** and **7**. But others, such as the signalling lipids or acylcarnitines we measured in **Chapter 3,** are poorly understood and do not directly reflect a biological pathway. Metabolomic pathway analysis can help to improve biological interpretation, but such analyses require a relatively large volume of metabolites. The targeted metabolomics panel of 89 metabolites we measured in Chapter 3 was insufficient for such an analysis. Hence, using a more extensive metabolite platform or untargeted metabolomics analysis for metabolomic pathway analysis may help the biological interpretation.

When used for investigating the effects of dietary interventions, biological interpretation is additionally complicated by the fact that many circulating metabolites can reflect both dietary intake as exposure biomarkers and dietary effect or health biomarkers from endogenous metabolism.[89] For example, in **Chapter 7,** we found that the LFHP diet decreased plasma MUFA fraction, while, not surprisingly, the HMUFA diet increased plasma MUFA fraction. Higher relative concentrations of plasma MUFA have been associated with increased risk of future T2DM in observational studies[14,90,91] and interpretation of a change in plasma MUFA concentrations can thus be ambiguous.

We mainly measured plasma metabolites to explore hepatic lipid metabolism (**Chapters 3, 5** and **7**). While these metabolites can give us leads on possible underlying biological processes involved in the differences between liver and muscle IR (**Chapter 5**) or in diet-induced health effects (**Chapters 3** and **7**), more complex methods are required to investigate the different biological processes of hepatic metabolism that result in such plasma metabolite concentrations. Since the liver is practically inaccessible in humans, measurement of *in vivo* hepatic metabolism relies on indirect measures. Various aspects of hepatic lipid metabolism can be studied with stable-isotope methodologies, including DNL, hepatic and whole-body fat oxidation, VLDL kinetics, and the source of fatty acids in VLDL.[92] The fatty acid composition of liver fat and the fatty acid sources of liver fat can be studied by combining tracer methods with advanced imaging techniques or taking liver biopsies. However, the latter is ethically only allowed in individuals undergoing surgery and thus only possible for some research.[92,93] Nevertheless, like the clamp method, such methods are expensive, labour-intensive and burdensome.

Fasting versus dynamic metabolite measures

Measuring circulating metabolites in response to a meal may be a more sensitive measure of metabolic health than fasting metabolites. A meal provides a metabolic stressor to the body that requires a coordinated response from the gut, liver, pancreas, skeletal muscle, and adipose tissue to regain homeostasis. Hence, postprandial metabolite levels reflect the complex interplay between the key metabolic organs, and may therefore provide more insights into the functioning of these organs

than circulating metabolites in the fasting state, which are tightly controlled.[94] Our findings support the use of meal challenge tests as a more sensitive measure of metabolic perturbations. For instance, we observed that most individuals with tissue-specific IR or combined muscle and liver IR had fasting glucose levels in the normoglycemic range (**Chapter 4**). The impairments in glucose homeostasis only became apparent when measuring glucose and insulin responses to an OGTT. Similarly, individuals with liver or muscle IR had similar fasting blood lipid profiles, but very distinct postprandial lipid responses to a high-fat mixed meal (**Chapter 5**). In addition, while fasting blood lipid measurements suggested that improved hepatic lipid metabolism may have mediated observed phenotype-diet interactions (**Chapter 6**), extensive postprandial metabolite profiling revealed that improvement of postprandial lipid metabolism was mainly diet-specific (**Chapter 7**), and hence, likely not the primary mediator of the observed phenotype-diet interactions.

Studying postprandial responses also comes with several challenges. Firstly, the biological interpretation is not always straightforward. For many metabolites in the Nightingale platform, for instance, which we used in **Chapters 5** and **7**, a(n) (un) healthy postprandial response has yet to be defined, and differences in or changes in postprandial responses are thus difficult to interpret. Secondly, since the meal composition affects the postprandial response, postprandial responses cannot be readily compared across studies due to different meals being used. Thirdly, the statistical analysis of postprandial responses is rather complicated. Postprandial responses can be quantified in many ways, using the total area under the curve (AUC), incremental AUC (iAUC), or by calculating derivatives such as the slope or peak value.[95] In addition, the actual curves can be analysed using repeated measures analysis. All methods give slightly different information about the postprandial response and have pros and cons. While repeated measures analysis considers all information about the curve shape and dynamics, it may fail to detect more subtle yet relevant differences or changes that can be picked up with, for instance, the iAUC. The best method to quantify and analyse postprandial responses depends on the research question and probably also on the metabolite. However, for most metabolites, we do not know the most relevant feature of their postprandial response.

To summarise, measuring circulating metabolites only provides part of the biological picture. For in-depth mechanistic insights, animal studies or advanced *in vivo* tracer and imaging methods are required. Nevertheless, the measurement of metabolites in blood, preferably dynamic measurements, is a relatively low invasive and easy alternative for exploration of leads towards possible underlying mechanisms of diet-induced health effects. In addition, for *post-hoc* analyses of completed trials, (metabolomics) analysis of collected samples is usually the only option.

Concluding remarks

The research in this thesis demonstrates for the first time that fine-tuning the current 'one-size-fits-all' dietary guidelines according to tissue-specific insulin resistance may provide additional health benefits for individuals with overweight or obesity, independent of weight loss. Our findings indicate that individuals with predominant muscle IR would benefit more from a diet low in fat and rich in protein and fibre for improving insulin sensitivity, while individuals with predominant liver IR would benefit more from a diet rich in MUFA. Interestingly, further characterisation of the intervention effects in this study using plasma metabolomics revealed diet-specific effects independent of IR phenotype: consumption of the low-fat, high-protein, high-fibre diet resulted in greater improvements in postprandial lipid metabolism as compared to consumption of the high-MUFA diet in both IR phenotypes. Therefore, individuals with liver IR and concurrent dyslipidemia might benefit more from a low-fat, high-protein, high-fibre diet by improving postprandial lipid metabolism, whereas normolipidemic individuals with liver IR might benefit more from a high-MUFA diet by improving glucose homeostasis. These findings illustrate the need for further refinement of metabolic phenotypes and personalised dietary advice, such as lipemic measures, to optimise the cardiometabolic health benefits of precision nutrition.

Evidently, biology-based precision nutrition is still in its infancy. Our work provides the first trial evidence for the potential of precision nutrition based on metabolic phenotype as a strategy for improving cardiometabolic health. The research in this thesis also highlights the complex interplay between metabolic heterogeneity and diet. In that respect, our findings may have provided few answers but certainly generated many new questions, thereby providing leads for future research.

Future perspectives

In this thesis, we identified various leads towards possible mechanisms underlying the observed metabolic heterogeneity in tissue-specific IR, diet-induced health effects, and their interactions. More mechanistic insights would help to further refine precision nutrition strategies by better targeting specific biological processes or metabolic perturbations. Diet-induced effects on sAT inflammation, systemic inflammation, and immunometabolism can be explored by transcriptomic and proteomic analyses of collected sAT biopsies and PBMCs, as well as flow cytometry to assess immune cell composition. The sAT transcriptome and proteome may additionally provide more insights into the hypothesised diet-induced effects on adipose tissue health, lipolysis, and BCAA metabolism. Since skeletal muscle is the primary site of BCAA catabolism, transcriptomic and proteomic analyses in collected

skeletal muscle biopsies are also warranted. Furthermore, future studies using stable-isotope methodologies are required to gain a better understanding of hepatic lipid metabolism (perturbations) in tissue-specific IR, diet-induced effects on hepatic lipid metabolism, and their interaction.

While we showed that tissue-specific IR is a valuable target for precision nutrition, refinement of metabolic phenotyping by including other factors that may be relevant for dietary response and health outcomes will likely further optimise the health benefits of precision nutrition. In addition, the majority of individuals with overweight or obesity does not have tissue-specific IR; numbers depend on the used definition or cut-off, but in our screening population, 40% had no liver nor muscle IR, and 28% had combined liver and muscle IR (**Chapter 4**). Identification of other metabolic phenotypes relevant for dietary response would enable the use of precision nutrition for a broader population. Compared to postprandial glycemic responses, postprandial lipid responses are even more variable between individuals, are less determined by genetics and more determined by individual, modifiable factors, and may therefore be a relevant additional factor to phenotype individuals on.[69] Our findings suggest that postprandial lipids, BCAA, and β-hydroxybutyrate may be involved in phenotype-diet interactions. Therefore, postprandial responses of these metabolites may be a relevant additional factor to phenotype individuals on. Population-specific cut-offs or clustering analysis can be used in existing data of mixed-meal responses to identify metabolic subgroups based on postprandial glucose, insulin, lipid, and other metabolite dynamics in response to a mixed meal. Long-term dietary responses of these metabolic phenotypes could subsequently be predicted by combining existing knowledge from dietary intervention trials and mechanistic studies with predictive AI approaches in datasets from completed trials. The potential of basing precision nutrition on such newly defined metabolic phenotypes should be tested in a clinical trial.

The determination of metabolic phenotype should be simple, quick, and affordable to allow for its use in large-scale studies or clinical practice. In addition, phenotypes are not static, and if a precision nutrition intervention is successful, it will likely affect the very parameters on which the intervention was based. Hence, periodic re-evaluation is desirable. New biomarkers, simplified indices, or metabolite signatures are thus needed for metabolic phenotyping. OGTTs with fewer blood drawings combined with measurement of fasting blood lipids and body fat already show promising results for estimating hepatic insulin resistance,[96] a method that may be used in the clinic. For at-home measurements, new surrogate markers may be developed using CGM[97] in combination with, for instance, DBS for lipids and C-peptide. The development of technologies that can frequently or continuously measure metabolites other than interstitial glucose, such as lipids or insulin, at home with minimal invasiveness would facilitate easier phenotyping. Once we have simplified methods for determining metabolic phenotype, precision nutrition based on these metabolic phenotypes can be tested on a large scale in a field study.

Lastly, biology-based precision nutrition may eventually be perfectly suited to one's biology, but personalised dietary advice is only effective if applied in daily life. In that respect, personalisation of dietary advice may already encourage dietary modification.[98] Still, other factors are at least equally important for promoting long-lasting dietary changes, including dietary preferences and socio-economical, cultural, and psychological factors.[99,100] Moreover, additional features that affect dietary responses include sleep, previous meals, physical activity, meal sequence, and meal timing.[69,101] Maximal health benefits are likely to be achieved by integrating these features for personalised dietary advice, for instance, in a virtual digital twin. In such a virtual model, personal data and preferences are combined with real-time monitoring of, for example, dietary intake, sleep, physical activity, and glycemic responses with wearable sensors to provide personalised nutritional advice that is predicted to induce the greatest health benefits.[100]

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APPENDICES

General summary

A growing proportion of worldwide morbidity and mortality is attributable to poor diet. Suboptimal diet quality increases the risk of cardiometabolic diseases by promoting intermediate risk factors such as high blood pressure and elevated blood glucose, triglyceride, and cholesterol levels, or can impair metabolic health via the development of adiposity, which is upstream of most cardiometabolic diseases. Improving diet quality is therefore a great strategy for the prevention of cardiometabolic diseases. However, the mechanisms that underlie diet-induced health effects are often poorly understood. Besides, there is great inter-individual variation in how people respond to foods, meals, or diet, and emerging evidence indicates that metabolic heterogeneity may partly account for that variation. A better characterisation of metabolic heterogeneity and a better understanding of the mechanisms by which dietary modification affects metabolic health may provide leads for more successful dietary interventions that better target these metabolic impairments. The research in this thesis aimed to contribute to a better understanding of the role of metabolic heterogeneity in response to diet, with a specific focus on tissue-specific insulin resistance. In addition, we aimed to contribute to a better understanding of the mechanisms by which diet affects metabolic health by investigating circulating metabolites related to cardiometabolic health and liver health or function.

FGF21 is a hepatokine that is involved in energy and nutrient metabolism. Its metabolic regulation and physiological functions are highly complex and poorly understood, but circulating FGF21 levels have been proposed to be a marker of metabolic health status. Our group previously demonstrated that a weight loss diet with 25% energy restriction and high nutrient quality resulted in greater weight loss and a more anti-atherogenic blood lipid profile than a diet with similar energy restriction but low nutrient quality, while both diets reduced insulin resistance and liver fat to a similar extent.6 In **Chapter 2**, we aimed to explore whether circulating FGF21 levels are a marker of metabolic health by investigating whether these health improvements were reflected by changes in circulating FGF21 levels. We found that both diets did not affect fasting or postprandial plasma FGF21 levels, nor did overall energy restriction. We concluded that changes in plasma FGF21 are not a sensitive marker of diet-induced changes in metabolic health in relatively healthy overweight individuals.

Fatty liver is an increasingly prevalent condition that is closely associated to obesity and insulin resistance. Our group previously demonstrated that a 12-week refined wheat intervention modestly increased liver fat (+1.5% percentage points), whereas whole-grain wheat did not affect liver fat content in overweight individuals with mildly elevated cholesterol levels. In **Chapter 3**, we aimed to explore potential underlying mechanisms of the preventive effect of whole-grain wheat consumption or

the detrimental effect of refined wheat consumption on liver fat accumulation. To that end, we investigated the effects of these wheat interventions on plasma metabolites involved in lipid metabolism, including betaine, choline, acylcarnitines, bile acids, and signalling lipids. We found that the wheat interventions did not robustly affect these circulating metabolites. These findings suggest that the effects of the wheat interventions on liver fat accumulation were not likely to be mediated by the increased intake of betaine and choline from whole-wheat grain or by effects on bile acid metabolism.

The pathophysiology of whole-body insulin resistance is characterised by great heterogeneity, with inter-individual differences in the development of insulin resistance in the various metabolic organs, including the liver and skeletal muscle. This metabolic heterogeneity may be a target for precision nutrition strategies. For the first time, we prospectively studied in a randomised trial whether individuals with tissue-specific insulin resistance benefit from different diets (the PERSON study). The design, rationale, and preliminary screening results of the PERSON study are described in **Chapter 4**. In **Chapter 6**, we demonstrated that individuals with predominant muscle insulin resistance (muscle IR) benefitted more from a 12-week low-fat, high-protein, and high-fibre (LFHP) diet, while individuals with predominant liver insulin resistance (liver IR) had greater improvements in peripheral insulin sensitivity, glucose tolerance, fasting serum triglycerides (TAG), and C-reactive protein upon a high-MUFA (HMUFA) diet. No effects were observed on the primary outcome disposition index. These findings indicate that precision nutrition based on metabolic phenotype may be superior to a one-size-fits-all diet based on general dietary guidelines for improving cardiometabolic health.

In **Chapter 7**, we further characterised the effects of the LFHP and HMUFA diets on fasting and postprandial plasma metabolite profile to identify leads towards potential underlying mechanisms of the differential effects of HMUFA and LFHP diets in tissue-specific IR. We demonstrated that the greater reduction in fasting TAG in the phenotype-diet combinations observed in **Chapter 6** was due to a larger decrease of TAG in hepatically derived VLDL particles, and not due to changes in TAG in the other lipoproteins. Furthermore, we showed that overall, irrespective of IR phenotype, the LFHP diet resulted in larger reductions in fasting and postprandial plasma concentrations of small HDL particles and almost all subclasses of VLDL particles, TAG fractions in VLDL, small LDL, and HDL, β-hydroxybutyrate, and postprandial BCAA concentrations as compared to the HMUFA diet. We concluded that the observed phenotype-diet interactions in **Chapter 6** might be related to diet-induced effects on the delivery of lipids to the liver or hepatic lipid storage or secretion. Nevertheless, a diet low in fat and rich in protein and fibre may be more effective for improving postprandial lipid and BCAA metabolism than a diet rich in MUFA for both individuals with liver or muscle IR. Therefore, individuals with liver IR and concurrent dyslipidemia might benefit more from a low-fat, high-protein, high-fibre diet to improve postprandial lipid metabolism. In contrast, normolipidemic individuals with liver IR might benefit more from a high-MUFA diet resulting in improved glucose homeostasis.

In **Chapter 5**, we aimed to better characterise metabolic heterogeneity with respect to fasting and postprandial metabolism in tissue-specific insulin resistance. To that end, we compared the postprandial plasma metabolome in response to a high-fat mixed meal between individuals with muscle IR or liver IR in a cross-sectional analysis of the PERSON study. We demonstrated that liver IR was characterised by a more dyslipidemic postprandial metabolite profile compared to muscle IR, with greater increases in postprandial large VLDL particles and triglycerides in several VLDL, LDL, and HDL subclasses in individuals with liver IR after consumption of a high-fat mixed meal. This work points towards more pronounced impairments in postprandial lipid metabolism in liver compared to muscle IR.

The research in this thesis demonstrates for the first time that fine-tuning the current 'one-size-fits-all' dietary guidelines according to tissue-specific insulin resistance may provide additional health benefits for individuals with overweight or obesity, independent of weight loss. Hence, our findings support the potential of precision nutrition based on metabolic phenotype for improving metabolic health. Our work also illustrates the need for further refinement of metabolic phenotypes and customised dietary advice, such as lipemic measures, to optimise the cardiometabolic health benefits of precision nutrition. The findings in this thesis have provided new leads on possible mechanisms involved in diet-induced health effects, which can support the development of future precision nutrition strategies for improving metabolic health.

Dutch summary | Nederlandse samenvatting

De stijgende levensverwachting van de afgelopen 30 jaar gaat niet gepaard met een evenredige verbetering in gezondheid: wereldwijd leven mensen langer, maar die jaren worden steeds vaker in slechte gezondheid doorgebracht. Deze ziektelast is in belangrijke mate toe te schrijven aan cardiometabole risicofactoren zoals overgewicht, hoge bloeddruk, verhoogd cholesterol, insulineresistentie (de verminderde gevoeligheid van cellen in het lichaam op insuline, het hormoon dat de bloedsuikerspiegel regelt), en hoge bloedsuiker, welke het risico op chronische aandoeningen zoals hart- en vaatziekten en diabetes type 2 verhogen. Ongezonde voeding is één van de voornaamste oorzaken van dergelijke risicofactoren. Één op de vijf sterfgevallen wereldwijd is naar schatting te wijten aan slechte eetgewoonten. Het bevorderen van gezonde voeding is daarom cruciaal voor de preventie van cardiometabole ziekten en verlaging van wereldwijde ziekte en sterfte.

Niet iedereen reageert echter hetzelfde op voeding; het meest gunstige voedingspatroon voor cardiometabole gezondheid verschilt mogelijk per persoon. Deze variatie lijkt deels toe te schrijven aan metabole heterogeniteit, oftewel verschillen in stofwisseling tussen individuen. Verder weten we vaak nog niet precies welke mechanismen ten grondslag liggen aan de gunstige effecten van gezonde voeding op cardiometabole gezondheid. Een betere karakterisering van verschillen in stofwisseling tussen individuen en meer inzicht in hoe voeding de cardiometabole gezondheid beïnvloedt, kunnen aanknopingspunten bieden voor meer passende en effectieve voedingsprogramma's ter preventie van cardiometabole aandoeningen. Het doel van dit proefschrift was om meer inzicht te verkrijgen in 1) de rol van metabole heterogeniteit in de respons op voeding, en 2) de mechanismen waardoor voeding de cardiometabole gezondheid beïnvloedt.

FGF21 is een signaalstof die geproduceerd wordt door de lever en een belangrijke rol speelt in de regulatie van de stofwisseling. Hoewel de precieze biologische functies van FGF21 grotendeels onbegrepen zijn, wijst onderzoek uit dat FGF21-concentraties in het bloed mogelijk een marker zijn van de metabole gezondheidsstatus. Eerder onderzoek van onze onderzoeksgroep heeft aangetoond dat een afvaldieet met een hoge kwaliteit van voedingsstoffen na 12 weken tot meer gewichtsverlies en een grotere afname in bepaalde ongunstige vetten in het bloed resulteerde dan een afvaldieet met vergelijkbare caloriebeperking maar een lage kwaliteit van voedingsstoffen. In vervolgonderzoek hebben we onderzocht of deze gezondheidsverbeteringen gepaard gingen met veranderingen in FGF21-concentraties in het bloed met het doel om te onderzoeken of FGF21 in het bloed een marker is voor metabole gezondheid (**hoofdstuk 2**). We vonden dat beide afvaldiëten geen invloed hadden op de nuchtere of postprandiale (na een maaltijd) FGF21-concentratie in het bloed. Veranderingen in FGF21 in het bloed lijken dus geen gevoelige marker zijn voor dieet-geïnduceerde veranderingen in metabole gezondheid bij relatief gezonde mensen met overgewicht.

Leververvetting is een steeds vaker voorkomende aandoening die nauw verband houdt met overgewicht en insulineresistentie. Onze onderzoeksgroep heeft eerder aangetoond dat de consumptie van witbrood gedurende 12 weken de hoeveelheid vet in de lever verhoogde bij personen met overgewicht en licht verhoogde cholesterolwaarden, terwijl volkorenbrood de hoeveelheid vet in de lever niet beïnvloedde. In vervolgonderzoek hebben we mogelijke onderliggende mechanismen onderzocht van het preventieve effect van het consumeren van volkoren tarwe of het ongunstige effect van het consumeren van geraffineerde tarwe op vetophoping in de lever (**hoofdstuk 3**). Hiertoe hebben we de effecten van deze tarwe-interventies onderzocht op metabolieten in het bloed die betrokken zijn bij de vetstofwisseling, waaronder betaine, choline, acylcarnitines, en galzuren. We vonden dat de tarwe-interventies geen robuust effect hadden op deze circulerende metabolieten. Deze bevindingen geven aan dat de effecten van de tarwe-interventies op de vetophoping in de lever waarschijnlijk niet werden veroorzaakt door een verhoogde inname van betaïne en choline uit volkoren tarwe of door effecten op de galzuurstofwisseling.

Insulineresistentie kan voorkomen in alle metabole organen: spieren, lever, en vetweefsel. De ontwikkeling van insulineresistentie in de verschillende organen kan echter verschillen tussen individuen, wat weefselspecifieke insulineresistentie wordt genoemd. Het afstemmen van voedingsinterventies op weefselspecifieke insulineresistentie is mogelijk een effectieve strategie ter verbetering van de metabole gezondheid. We hebben voor het eerst onderzocht met gerandomiseerde onderzoek of personen met weefselspecifieke insulineresistentie baat hebben bij verschillende voedingspatronen (de PERSON-studie). Het ontwerp, de rationale en tussentijdse screeningresultaten van de PERSON-studie zijn beschreven in **hoofdstuk 4**. Ons onderzoek wees uit dat mensen met insulineresistentie in de spieren (spier-IR) meer baat hadden bij een voedingspatroon relatief laag in vet en rijk aan eiwit en vezels (LFHP), terwijl mensen met insulineresistentie in de lever (lever-IR) grotere verbeteringen hadden na 12 weken een voedingspatroon rijk aan enkelvoudig onverzadigde vetzuren (HMUFA) gevolgd te hebben wat betreft insulinegevoeligheid, glucosehuishouding, en vetten en ontstekingsmarkers in het bloed (**hoofdstuk 6**). Deze bevindingen geven aan dat gepersonaliseerde voeding of 'precision nutrition' gebaseerd op IR-subtype mogelijk effectiever is voor het verbeteren van de cardiometabole gezondheid dan een 'one-size-fits-all'-voedingspatroon op basis van de algemene voedingsrichtlijnen.

Vervolgens hebben we de effecten van de LFHP- en HMUFA-voedingspatronen op metabolieten in het bloed verder gekarakteriseerd met het doel om aanknopingspunten te identificeren voor mogelijke onderliggende mechanismen van de verschillende effecten van de HMUFA- en LFHP-voedingspatronen bij personen met spier-IR of lever-IR (**hoofdstuk 7**). We hebben de metabolieten in bloed gemeten in zowel de nuchtere toestand, als na de consumptie van een vet- en suikerrijke maaltijd. We vonden dat de in hoofdstuk 6 gevonden grotere afname van bepaalde vetten in het bloed in de spier-IR/LFHP en lever-IR/HMUFA groepen te wijten was aan een grotere afname van triglyceriden in VLDL-deeltjes die door de lever geproduceerd worden, en niet aan veranderingen in triglyceriden in andere lipoproteïnen. Verder vonden we dat over het algemeen, ongeacht het IR-subtype, het LFHP-voedingspatroon resulteerde in grotere afnames in bepaalde vetten en aminozuren in het bloed, zowel in nuchtere toestand als na de maaltijd, in vergelijking met het HMUFA-voedingspatroon. Deze bevindingen wijzen erop dat de waargenomen IR-subtype-voedingspatroon-interacties in hoofdstuk 6 mogelijk gerelateerd zijn aan interventie-geïnduceerde effecten op vetmetabolisme in de lever. Een voedingspatroon laag in vet en rijk aan eiwitten en vezels lijkt echter effectiever voor het verbeteren van postprandiaal vet- en aminozuurmetabolisme dan een voedingspatroon rijk aan MUFA, voor zowel personen met lever- als spier-IR. Daarom zouden personen met lever-IR en een verstoord vetmetabolisme mogelijk meer baat hebben bij een laag-vet, hoog-eiwit, hoog-vezel voedingspatroon door verbetering van het postprandiale vetmetabolisme, terwijl personen met lever-IR en zonder verstoord vetmetabolisme mogelijk meer baat hebben bij een voedingspatroon rijk aan MUFA dat de glucosehuishouding verbetert.

Eerder onderzoek heeft laten zien dat weefselspecifieke insulineresistentie gepaard gaat met metabole heterogeniteit wat betreft nuchter metabolietenprofiel in het bloed. In **hoofdstuk 5** beoogden we de metabole heterogeniteit in weefselspecifieke insulineresistentie verder te karakteriseren. Hiertoe hebben we het metabolietenprofiel in het bloed in respons op een vet- en suikerrijke maaltijd vergeleken tussen individuen met spier-IR of lever-IR in een cross-sectionele analyse van de PERSON-studie. We vonden dat mensen met lever-IR een grotere stijging hadden in grote VLDL-deeltjes en triglyceriden in verschillende VLDL-, LDL-, en HDL-deeltjes in het bloed na de maaltijd, in vergelijking met personen met spier-IR. Dit werk wijst erop dat lever-IR gekenmerkt wordt door grotere verstoringen in postprandiaal vetmetabolisme dan spier-IR.

Het onderzoek in dit proefschrift toont voor de eerste keer aan dat het verfijnen van de huidige algemene voedingsrichtlijnen volgens weefselspecifieke insulineresistentie mogelijk verdere gezondheidswinst kan opleveren voor personen met overgewicht of obesitas, onafhankelijk van gewichtsverlies. Onze bevindingen ondersteunen daarom 'precision nutrition' op basis van metabool subtype als veelbelovende strategie voor het verbeteren van cardiometabole gezondheid. Daarnaast illustreert ons werk dat verdere verfijning van metabole subtypen en op maat gemaakt voedingsadvies mogelijk de effectiviteit van 'precision nutrition' verder kan optimaliseren, bijvoorbeeld op basis van biomarkers van vetmetabolisme in het bloed. De bevindingen in dit proefschrift hebben verder nieuwe aanknopingspunten opgeleverd voor mechanismen die mogelijk betrokken zijn bij de effecten van voeding op cardiometabole gezondheid. Deze bevindingen kunnen bijdragen aan de ontwikkeling van toekomstige 'precision nutrition'-interventies ter preventie van diabetes type 2 en andere chronische aandoeningen.

Acknowledgements | Dankwoord

I would like to express my sincere gratitude to everyone who contributed directly or indirectly to the research presented in this thesis and my PhD years in general.

Lydia, ik kan je niet genoeg bedanken voor jouw begeleiding, vertrouwen, en enthousiasme gedurende de afgelopen jaren. Jouw sturing en creativiteit waren onmisbaar, en ik waardeer ook jouw aandacht voor alles in het leven buiten werk. Ik vind het ontzettend leuk dat we in ieder geval komend jaar nog samenwerken aan mooie projecten.

Edith, bedankt voor jouw kundige en kritische bijdragen aan met name de opzet- en uitvoeringsfases van de PERSON-studie, en aan mijn manuscripten. Ik ben dankbaar voor jouw betrokkenheid en interesse in mijn projecten, en jouw bereidheid om altijd mijn vragen te beantwoorden, ondanks de vele andere projecten waar je bij betrokken bent.

Aan de commissieleden - prof. Walter Gerrits, prof. Baukje de Roos, prof. Mireille Serlie en dr. Renée de Mutsert - heel hartelijk dank voor het lezen en beoordelen van mijn proefschrift, en voor jullie bijdrage aan mijn verdediging.

Lieve mensen van de HRU, bedankt voor jullie grootse toewijding en de gezelligheid. Soms voelde het alsof we vanwege de COVID-lockdowns en andere uitdagingen het einde van de studie nooit zouden halen, maar uiteindelijk is het ons toch maar mooi gelukt!

Els, bedankt voor jouw enorme bijdrage aan alles omtrent de voedingsinterventie. Jouw directheid, nuchterheid en humor maken samenwerken met jou een groot plezier. Henriëtte, jouw positieve instelling, inventiviteit en flexibiliteit hebben ons niet alleen geholpen bij de planning, logistiek en uitvoering van de studie, maar waren ook van onschatbare waarde voor het welzijn en werkplezier van het hele team. Bedankt voor alle inzet, vrolijkheid en goede zorgen - zelfs de 600e OGTT was dankzij jullie een feestje.

Koen, waar moet ik beginnen? Duizendmaal dank voor al je onbaatzuchtige hulp met zo ongeveer alle praktische aspecten van de studie. Ik ben ook waanzinnig dankbaar dat je zelfs na je tijd als onderzoeksassistent op het project nog de MRI-metingen hebt uitgevoerd. Dankjewel!

Odette en Meeke, bedankt voor jullie kundige en warme begeleiding van de proef-personen. De lage drop-out en hoge compliance hebben we grotendeels aan jullie

fijne begeleiding te danken! Judith, bedankt voor je begeleiding van de proefpersonen in het begin van de studie.

Mechteld, bedankt voor je onvermoeibare inzet en enthousiasme met betrekking tot al het labwerk. Ineke, bedankt voor je medische expertise en het meedenken gedurende de studie. Het spijt me dat de PERSON-studie een van je laatste studies was, ik hoop dat je het me inmiddels vergeven hebt. Myrthe, wat werk jij heerlijk georganiseerd en efficiënt, bedankt voor al je bijdragen aan de studie. Anne W-vdK, bedankt voor alle gezellige ochtenden! Diana, cannule-koningin, bedankt voor je hulp tijdens al die testdagen. Rav, bedankt voor je uitstekende gastheerschap en hulp met data-invoer. Desiree, bedankt voor het inspringen, je hebt ons meerdere malen uit de brand geholpen. Laura, bedankt voor je hulp met de testdagen in het laatste jaar van de studie.

Colleagues from Maastricht, thank you for the great collaboration and interesting discussions. Kelly en Inez, wat was het fijn om lief en leed met betrekking tot de studie met jullie te delen. Jullie doorzettingsvermogen is echt bewonderenswaardig, met zowat elke dag een testdag. Ontzettend knap ook hoe jullie bijna alles zelf hebben gedaan, van het afnemen van biopten tot de voedingsbegeleiding van de deelnemers. Het was een plezier om met jullie samen te werken! Ellen en Gijs, jullie ook bedankt voor de prettige samenwerking, interessante discussies en altijd constructieve feedback. Ik heb veel van jullie geleerd. Gabby, ook jij was onmisbaar voor de studie. Bedankt voor al je praktische ondersteuning en alles met betrekking tot de data. Suzanne and Dilemin, thank you for your contributions to the study. Balázs, thank you for our nice chats and your help with data analysis.

Lisa (Radboudumc), thank you so much for helping out with the ActivPALs and the MRI measurements. I'm glad you managed to use the data for your own thesis after all. Mariëlle, bedankt voor je samenwerking in het TiFN-consortium en je hulp met de MTPRT. Other members of the TiFN consortium – Ardy, Axelle, Bart, Cécile, Dick, Ilja, Jan, Jeanne, Jennifer, Johan, Jordi, Julia, Maaike, Matthijs, Michiel, Olof, Peter, Rodrigo, Sanne, Szabi – many thanks for the interesting discussions and your valuable contributions to this work.

Marco, bedankt voor je hulp met de MRI-trainingen en de image analyses. Paul, dank voor het opzetten en optimaliseren van het scanprotocol voor de nieuwe MRI-scanner. Arjen (ZGV) en Vera (UM), bedankt voor jullie hulp met het protocol. Anne vdW, bedankt voor al je hulp met de werving, Biobank en Traqq. Corine, Arabella en Hanne, bedankt voor alle diëtetiek-ondersteuning. Pieter, Julia, Kaj, Babette en Myrthe vdH, bedankt voor jullie hulp met het invoeren van data. Adrienne en collega's

van het secretariaat – Jasmijn, Gea, Sjanneke, Riekie, Jeanine, Karen – dank voor jullie ondersteuning.

To all students that helped with the data collection - Yurike, Fenna, Pauline, Emma, Lotte, Lieke, Donovan, Nicky, Sanne, Anne, Eline, Bas, Monique, Shah, Ruth, Bonnie, Samantha, Laura, Christina, Ricky, Fardau, Vivian, Maaike, Bianca, Anna, Kaat, Nigel, Louise, Anita, Femke Fleur, Sharon, Anouk, and Sofia - many thanks for all your hard work!

Aan alle deelnemers van de PERSON-studie: bedankt voor jullie inzet en toewijding. Deelname aan dit onderzoek was niet makkelijk, we hebben veel van jullie gevraagd. Wetenschappelijk onderzoek zou niet mogelijk zijn zonder dappere deelnemers zoals jullie!

Charlotte, jouw gezelligheid en humor maken je een geweldige kantoorgenoot en wat heb ik je gemist nadat de pandemie uitbrak! Ik vind het heel bijzonder dat je zo kort na de geboorte van Olivier mijn paranimf wil zijn, dankjewel. Lisa, onze gangmaker en in-house beeldbewerker, bedankt voor alle feestjes, diners, je Photoshop-skills en je bereidheid om samen mijn lievelingslied te zingen in een Spaanse karaokebar. Heel gezellig dat je mijn paranimf bent, dankjewel.

Voormalige roomies – Xanthe, Brecht en Judith – bedankt voor de fijne en verrijkende gesprekken, goede adviezen en gezelligheid. Merel, jij zorgde ervoor dat ik me snel welkom voelde binnen de groep, dankjewel voor je oprechte interesse en goede zorgen. Tessa, bedankt voor de gezellige tijd in Denemarken en Canada! Mara, bedankt dat ik al die keren op jullie katten mocht passen; ik heb goede herinneringen aan mijn tijd met Coco en Soef in jullie fijne huis en zonnige tuin. Danny, thank you for all the nice talks, strange compliments and great food. NMG really is not the same without you. Frank, Patrick en de rest van het Stienstreet lab, bedankt dat jullie zulke fijne collega's zijn, jullie drive en enthousiasme zijn inspirerend. Monique, bedankt voor de gezelligheid en het altijd meedenken. Yan, thanks for the fun times in Spain!

Guido, Rinke, Sander, Wilma en Bryan, bedankt voor jullie waardevolle feedback en kritische vragen. Guido, altijd betrokken en behulpzaam, bedankt voor je belangstelling en adviezen. Sander, ik waardeer het heel erg dat je het mij toevertrouwde om op jullie huis te passen in de tropische zomer van 2019; wat een luxe om tijdelijk om de hoek van Helix te wonen. Shohreh and Mieke, thank you for the joy and laughter. Other (former) colleagues of the 2nd floor – Aina, Benthe, Diederik, Iris, Jenny, Jocelijn, Karin, Klaske, Marie-Luise, Mark, Marlies, Meirong, Michiel, Mingjuan, Miranda, Montse, Nhien, Philip, Rieneke, Roos, Roland, Roseanne, Ruolei, Shauna, Sophie, Suzanne, Susanne, Walid, Wout and Xiaolin – thank you for the good times!

Many thanks to the PhD Tour organisers - Arli, Elbrich, Moniek, Paulina, Pol, Rachelle & Vera - and all fellow participants for the great time in Canada. Also great fun: The Dinner Game. Veel dank aan de organisatoren - Adrienne, Mechteld, Guido, Koen en Pol – voor alle tijd en moeite die ze in dit fantastische, volledig uit de hand gelopen teamuitje hebben gestoken.

Lieve Eva, Irene, Jip, Lena, Mercedes, Milou, Nayra en Sara, onze inmiddels decennium- / decennialange (!) vriendschap is me heel dierbaar. Ondanks jullie lichte teleurstelling over dat ik mijn Wageningse werkdagen niet grotendeels doorbracht op een laboratorium om gehuld in labjas (en liefst ook nog met veiligheidsbril op) allerlei spannende experimenten uit te voeren (bij voorkeur in een erlenmeyer), toonden jullie altijd belangstelling in en enthousiasme over mijn werk. Dank voor jullie vriendschap, alle fijne tijd samen en jullie wijze woorden.

Mams en paps, bedankt voor jullie liefde, steun en vertrouwen. Wat bof ik met zulke lieve ouders. Lianne, allerliefste zus, bedankt voor onze onvoorwaardelijke band en alle plezier samen. Ik koester onze gedeelde liefde voor eurodance/trance/happy hardcore, karaoke en glitters zeer. Lieve oma, Mario, Sjon, Ramazan en Musa, dank voor de gezelligheid en jullie steun. Viv and Dan, thank you for the good times and your support. Also a special thanks to George and Ben for the joy they bring.

My dear Nicholas, thank you for everything.

Curriculum vitae

Anouk Gijbels was born on the 26th of October 1992 in Amsterdam. In 2010, she completed her secondary school education at the Montessori Lyceum Amsterdam. Her plans for spending a gap year backpacking through Asia were side-lined when she met a charming Liverpudlian and decided to move to Liverpool instead.

In 2012 - back in Amsterdam - Anouk undertook a Bachelor's degree in Health Sciences at the Vrije Universiteit Amsterdam, during which she did a minor in Nutrition & Health at Wageningen University. After graduating *cum laude* in 2015, Anouk returned to Wageningen University to pursue a Master's degree in Nutrition & Health. During her Master's thesis, she gained her first experience with human intervention studies by assisting in a clinical trial that tested the effects of a novel nutritional supplement on body composition and physical function in older adults with or at risk of undernutrition. Anouk completed her studies with a research internship at the Diabetes and Metabolism Research Group at Maastricht University, where she assisted with a clinical trial on the effects of resveratrol supplementation on insulin sensitivity in overweight adults. She graduated *cum laude* in 2017.

In September 2017, Anouk started as a PhD candidate in the Nutrition, Metabolism, and Genomics group at the Division of Human Nutrition and Health of Wageningen University, under the supervision of dr. Lydia Afman and prof. Edith Feskens. In close collaboration with Maastricht University, she performed one of the first randomised clinical trials in the field of precision nutrition to test the effects of a more personalised dietary intervention on cardiometabolic health outcomes. Anouk has presented her work at several national and international conferences and has co-supervised 27 BSc and MSc students during their thesis or internship.

From April 2023 onwards, Anouk continues to work on precision nutrition as a post-doctoral researcher in dr. Lydia Afman's group.

List of publications

Gijbels A*, Trouwborst I*, Jardon KM, Hul GB, Siebelink E, Bowser SM, Yildiz D, Wanders L, Erdos B, Thijssen DHJ, Feskens EJM, Goossens GH, Afman LA, Blaak EE. The PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) Study: Rationale, Design and Preliminary Screening Results. *Front Nutr.* 2021 Jun 30;8:694568. doi: 10.3389/fnut.2021.694568.

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Trouwborst I, Wouters K, Jocken JW, Jardon KM, **Gijbels A**, Dagnelie PC, van Greevenbroek MMJ, van der Kallen CJ, Stehouwer CDA, Schalkwijk CG, Richard N, Bendik I, Afman LA, Blaak EE, Goossens GH. Circulating and adipose tissue immune cells in tissue-specific insulin resistance in humans with overweight and obesity. *Obesity (Silver Spring).* 2023 Mar 30. doi: 10.1002/oby.23714.

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Expected publications

Trouwborst I, Jardon KM, **Gijbels A**, Hul GB, Feskens EJM, Afman LA, Linge J, Goossens GH, Blaak EE. Body composition in tissue-specific insulin resistance and in response to an isocaloric dietary macronutrient intervention. *Submitted*.

Sloun B, Goossens GH, Erdõs B, O'Donovan SD, **Gijbels A**, Trouwborst I, Jardon KM, Blaak EE, Afman LA, van Riel NAW, Arts ICW. Predicting the postprandial glucose response in tissue-specific insulin resistant phenotypes: a computational modeling approach. *Submitted*.

Gijbels A, Trouwborst I, Jardon KM, Goossens GH, Blaak EE, Feskens EJM, Afman LA. Hepatic insulin resistance and muscle insulin resistance are characterised by distinct postprandial plasma lipid profiles. *In preparation*.

Gijbels A, Jardon KM, Trouwborst I, Manusama KCM, Goossens GH, Blaak EE, Feskens EJM, Afman LA. Tissue-specific insulin resistance phenotype-diet interactions in fasting and postprandial metabolite responses to a 12-week high-MUFA or low-fat, high-protein, high-fibre diet: a secondary analysis of the PERSON study. *In preparation*.

Jardon KM, Umanets A, Venema K, **Gijbels A**, Trouwborst I, Hul GB, Afman LA, Goossens GH, Blaak EE. Gut microbiome profiling in tissue-specific insulin resistance: A cross-sectional analysis of the PERSON study. *In preparation*.

Jardon KM, Umanets A, Trouwborst I, **Gijbels A**, Hul GB, Feskens EJM, Venema K, Afman LA, Goossens GH, Blaak EE. The PERSON study: Effects of a 12-week metabolically targeted dietary intervention on the gut microbial profile in overweight and obesity. *In preparation*.

* Shared first authorship

Overview of completed training activities

Discipline-specific activities		
Conferences and symposia		
NASO Spring Meeting	NASO	Utrecht, 2018
DuSRA Annual Meeting	DuSRA	Leiden, 2018
Annual Dutch Diabetes Research Meeting	NVDO	Oosterbeek, 2018
NASO Spring Meeting	NASO	Utrecht, 2019
37th International Symposium on Diabetes and Nutrition | *poster*	DNSG	Kerkrade, 2019
Food Summit Personalized Nutrition	TiFN	Wageningen, 2019
Annual Nutrition Science Meeting 2020	ASN	virtual conference, 2020
Obesity: From Cell to Patient & Diabetes: Many Faces of the Disease	Keystone Symposia	virtual conference, 2021
DTMC Seminars	DTMC	virtual webinar, 2021-2023
NASO Spring Meeting	NASO	virtual conference, 2021
Annual Nutrition Science Meeting 2021	ASN	virtual conference, 2021
38th International Symposium on Diabetes and Nutrition| *poster*	DNSG	virtual conference, 2021
Challenges and Opportunities for Precision and Personalized Nutrition	NASEM Health and Medicine	virtual webinar, 2021
NASO Spring Meeting	NASO	Utrecht, 2022
Annual Nutrition Science Meeting 2022 | *poster*	ASN	virtual conference, 2022
Dutch Translational Metabolism Conference | *oral*	DTMC	Wageningen, 2022
NuGO week 2022 | *oral*	NuGO association	Tarragona, Spain, 2022
Annual Dutch Diabetes Research Meeting | *oral*	NVDO	Wageningen, 2022
Precision Nutrition in the prevention of chronic metabolic diseases | *oral*	TiFN project consortium	Maastricht, 2023
Courses		
Energy metabolism and body composition in nutrition and health research	VLAG	Wageningen, 2018
Basal Metabolism and Molecular Mechanisms in Diabetes	Danish Diabetes Academy	Nyborg, Denmark, 2019

General courses		
VLAG PhD week	VLAG	Baarlo, 2018
Philosophy and Ethics of Food Science and Technology	VLAG	Wageningen, 2018
Scientific Publishing	WGS	Wageningen, 2018
Supervising BSc and MSc thesis students	WGS	Wageningen, 2018
Pitch workshop	WUR | HNH	Wageningen, 2019
GCP training	WUR | HNH	Wageningen, 2019
Debating workshop	Jong-NAV, ENLP	Den Haag, 2019
Last stretch of the PhD programme	WGS	Wageningen, 2022
Writing propositions for your PhD	WGS	Wageningen, 2022
PhD workshop carousel	WGS	Wageningen, 2022
Introduction to R and R Studio	PE&RC	online, 2023

Assisting in teaching and supervision activities		
HNE-30706 Food Digestion: Nutrient Breakdown and Absorption	Course practical assistance	2018-2019
HNE-28305 Metabolic Aspects of Nutrition	Course practical assistance	2018-2020
HNH-34806 Applied Nutrigenomics	Course practical assistance, course work grading	2020-2021
Thesis or internship co-supervision of 1 BSc student and 26 MSc students		2018-2023

Other activities		
Preparation of research proposal	VLAG	Wageningen, 2017-2018
NMG group meetings	WUR | HNH	Wageningen, 2017-2023
HNH divisional staff seminars	WUR | HNH	Wageningen, 2017-2023
TiFN Retreat	TiFN	Arnhem, 2018
PhD study tour	WUR | HNH	Canada, 2019

Colophon

The research described in Chapters 4-7 of this thesis was organised by and executed under the auspices of TiFN, a public-private partnership on precompetitive research in food and nutrition. Funding for this research was obtained from DSM Nutritional Products, FrieslandCampina, Danone Nutricia Research, AMRA Medical AB, and the Top-sector Agri&Food. Other research described in this thesis was financially supported by the Division of Human Nutrition and Health at Wageningen University, the ZonMW ETH call (grant number 40-43500-98-4029), and the Topsector Agri and Food (TKI-AF 12083) and TNO roadmap Nutrition and Health, with co-funding by Cereal Partners Worldwide, the Dutch Bakery Center, and GoodMills Innovation GmbH.

Financial support from Wageningen University, the Netherlands Association for the Study of Obesity (NASO) and TiFN for printing this thesis is gratefully acknowledged.

Cover design, lay-out and print
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