Metabolites across the colorectal cancer continuum

A CLINICAL AND NUTRITIONAL PERSPECTIVE

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Metabolites across the colorectal cancer
continuum: *a clinical and nutritional
perspective*

Anne J.M.R. Geijsen

Thesis

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Chapter I

General introduction

The colorectal cancer continuum

The continuum of all cancers, including colorectal cancer, reflects the process in which normal cells progress via several steps into cancer cells. This multistep progression results from multiple genetic and/or epigenetic aberrations [1].

Benign colorectal tumors, adenomatous polyps, are formed from normal, healthy cells which can further develop into malignant colorectal tumors, see **Figure 1**. In stage I, the tumor has grown into the mucosa and submucosa of the intestinal wall. The cancer cells can further invade into the intestinal wall (stage II) and neighboring lymph nodes (stage III). Finally, malignant cells can travel through the circulation to distant lymph nodes and other distant tissues, causing metastases (stage IV) [2, 3].

In the following paragraphs the background on colorectal cancer, risk factors for colorectal cancer development, and potential risk factors influencing colorectal cancer recurrence and survival will be discussed from a clinical and nutritional perspective. The general introduction will highlight the potential role of metabolite concentrations in gaining more insight in the link between nutrition, metabolism, and the colorectal cancer continuum. Lastly, metabolomics approaches, the knowledge gap, and the objectives of this thesis will be discussed.

Colorectal cancer: a clinical perspective

The GLOBOCAN 2018 report of the International Agency for Research on Cancer (IARC) showed that colorectal cancer is among the top five most diagnosed cancer types in the world [4]. Worldwide, new colorectal cancer cases accounted for 10% of all newly diagnosed cancer cases in 2018 [4]. In the Netherlands, colorectal cancer was the second most diagnosed cancer, accounting for 11% of all newly diagnosed cancer cases in 2017 [5].

Symptoms of colorectal cancer are usually unspecific, including stomach ache, changed bowel movements, weight loss, and rectal bleeding [6]. Patients with complaints often visit their general practitioner relatively late, which can increase the chance of the diagnosis of a colorectal cancer stage [7, 8]. To anticipate on the low specificity of colorectal cancer complaints, population-wide screening has been initiated in numerous countries worldwide [9], including the Netherlands in 2014 [10, 11].

Figure 1 Overview of the colorectal cancer continuum

The cancer stage at diagnosis greatly determines colorectal cancer recurrence and survival [12-14]. All colorectal cancer stages combined showed a 5-year survival ranging from 60-70% in Western countries in the period 2010-2014 [15]. Stage I patients showed a 5-year survival of 91% and 88% for colon and rectal cancer, respectively, while the 5-year survival of stage IV patients was 13% and 14% for colon and rectal cancer, respectively [16]. Besides cancer stage, the tumor grade, age at diagnosis and treatment impact colorectal cancer survival [13]. Data on colorectal cancer recurrence after treatment is not routinely collected in individual countries or worldwide. It is estimated that 20-30% of curatively treated patients experience a colorectal cancer recurrence, of which 75% will occur within three years after the initial cancer diagnosis [17-19].

Colorectal cancer: a nutritional perspective

Around 60-65% of diagnosed colorectal cancer cases are sporadic, 20-25% are associated with having a family history of colorectal cancer and around 5-10% are linked to inherited genetic variations causing a higher lifetime risk of developing colorectal cancer, such as Lynch Syndrome and Familial Adenomatous Polyposis [20, 21]. Besides genetic factors, several other factors have been associated with colorectal cancer development. Age and sex are such factors, as colorectal cancer development increases after the age of 50 years and colorectal cancer incidence is higher among men compared to women [22]. The presence of inflammatory bowel disease and bacterial infections increase the risk of colorectal cancer, while the use of aspirin and the use of hormone therapy in postmenopausal women are protective factors for colorectal cancer risk [20, 22, 23].

Besides age, sex, genetic factors and bacterial infections, dietary and other lifestyle factors are considered to be main risk factors of colorectal cancer [20, 22, 24, 25]. Being overweight or obese and being taller are reported as convincing risk factors for colorectal cancer development [25]. Earlier studies also observed probable evidence for an increased colorectal cancer risk with increased smoking habits [20, 25]. Studies investigating the association between dietary factors and colorectal cancer risk focussed on nutrients, individual foods, food groups, as well as diet as a whole [26]. Macronutrients, i.e. total fat, carbohydrates, and protein, are not associated with colorectal cancer risk [25, 26]. When investigating micronutrients, a probable protective effect of dietary calcium and a suggestive protective effect of dietary vitamin D on colorectal cancer risk have been observed [25].

When evaluating individual food groups, probable evidence for a decreased risk of colorectal cancer has been reported for a high consumption of wholegrain products, fibre, and dairy products. Probable evidence for a high consumption of red meat and convincing evidence for a high consumption of processed meat and alcohol was observed for an increased the risk of colorectal cancer [25, 26]. Suggestive evidence is available for a potential protective effect of higher intake of fruits, vegetables [26], fish and products containing vitamin C [25] on colorectal cancer risk.

Diet quality indices and dietary patterns are used to assess exposure to combinations of food groups. Diet quality indices are calculated based on predefined recommendations, for example the World Cancer Research Fund (WCRF) score is based on the 2018 cancer prevention recommendations of the WCRF/American Institute for Cancer Research (AICR) [18]. Another example is the Dutch Healthy Diet index (DHD15-index), which is used to assess the adherence to the Dutch dietary guidelines of 2015 [19]. In addition to these indices, dietary patterns are constructed based on the reported dietary intakes of the study population and, therefore, data driven. Several a priori defined diet quality indices, such as the Healthy Eating Index, have been linked to colorectal cancer risk in the past [27]. Previous studies have also shown that better adherence to diet and other lifestyle-related guidelines to prevent cancer as recommended by the WCRF reduced cancer risk [28], including colorectal cancer [29-31]. Previous studies reported that a higher consumption of a Western dietary pattern is associated with a higher colorectal cancer risk, whereas a higher consumption of a Prudent diet has been associated with lower colorectal cancer risk [25]. A Western dietary pattern is generally regarded as “an unhealthy diet”, referring to high intakes of refined grains, sugary products (beverages, desserts, candy, etc.) red and processed meat and high-fat dairy products, and low intakes of vegetables and fruit. Contrary, a Prudent dietary pattern is characterized by healthy foods, such as high intakes of vegetables, fruits, whole-grain products and fish, and low intakes of meat [32]. In line with these findings, a higher intake of a Mediterranean diet, consisting of high intakes of legumes and nuts, fruits, vegetables, whole grains and a low consumption of meat and refined carbohydrates, has been associated with a lower colorectal cancer risk [33, 34].

In addition to dietary factors, other lifestyle factors have been linked to colorectal cancer risk [25]. A probable and suggestive protective effect on colorectal cancer risk has been reported for the use of calcium and multivitamin supplements, respectively [25]. However, a high intake of individual compounds in these multivitamin supplements, such as

folic acid have been associated with an increased colorectal cancer risk in earlier studies [35, 36]. Folic acid is the synthetic form of folate and, in addition to multivitamin supplements, is also present in folic acid-fortified foods. Folic acid is converted to the active form of folate by the enzyme dihydrofolate reductase (DHFR) [37]. Whenever folic acid cannot be converted to folate by DHFR, due to low DHFR activity or excessive intake of folic acid, unmetabolized folic acid may be detected in the circulation [37].

Folate plays an essential role in nucleotide synthesis as well as DNA repair and, indirectly, in the formation of the methyl donor S-adenosylmethionine involved in DNA methylation [38-40]. Folic acid has different biochemical properties compared to folate and is suggested to facilitate the growth of premalignant lesions or micrometastases once they have been established [35, 36, 41-43].

Since nutrition plays a key role in the etiology of colorectal cancer, it is important to establish whether dietary factors also play a role in colorectal cancer recurrence and survival. To date, studies investigating diet and colorectal cancer recurrence and survival are limited and further research is needed [44, 45]. Macro- and micronutrients and individual food groups in relation to colorectal cancer recurrence and survival have only been studied in few studies. Studies studying the association between red and processed meat [46-49] or alcohol [47, 50] and colorectal cancer outcomes have not shown consistent results, however higher intake of sugar-sweetened beverages [44] and high intakes of carbohydrates [51] may increase colorectal cancer mortality.

Two earlier studies suggested that a higher intake of a Western diet increased all-cause mortality risk and recurrence, while the opposite was observed for the Prudent diet [47, 52]. However, the reported associations were not statistically significant and were only present in patients receiving adjuvant therapy. Higher adherence to the Mediterranean diet and the healthy Nordic diet, reflecting a healthier diet, have been associated with better overall survival among colorectal cancer survivors [53]. Previous studies have suggested that better adherence to diet and other lifestyle-related guidelines to prevent cancer as recommended by WCRF reduced overall cancer mortality [28, 30, 54, 55], but they did not study the association with colorectal cancer individually.

Several longitudinal colorectal cancer patient cohort studies have been set up [56-58], collecting biospecimens and information about demographics, clinical characteristics, nutrition and lifestyle, to gain more knowledge about determinants of quality of life,

recurrence, and survival after colorectal cancer diagnosis. Analyzing data of these colorectal cancer patients allows to investigate the relationship between nutrition and colorectal cancer progression. The use of metabolomics in these ongoing studies may help to unravel the role of nutrition along the colorectal cancer continuum [59].

Metabolites across the colorectal cancer continuum

The interface between nutrition and other lifestyle factors and the colorectal cancer continuum can be explored by establishing the association between nutrition and metabolites. The complementary role of metabolites, in addition to questionnaire data, is relatively new in nutritional cancer epidemiology [60-65]. Metabolites refer to molecules present in biospecimens, including easily accessible specimens such as blood and urine, and are intermediates or end products of metabolism. The metabolic profile of an individual or the human metabolome, which is the totality of measured metabolites at sampling [66], reflects the biochemical pathways active at time of measurement [67]. Therefore, measuring metabolites is extremely valuable in providing information about the health status of a person, and metabolic changes associated with disease can help to get more insight in the underlying mechanisms of diseases, such as colorectal cancer, from a clinical and nutritional perspective.

Several studies focussing on metabolites have been conducted in the past to detect colorectal cancer [68, 69], as well as to gain insight in the underlying mechanisms leading to colorectal cancer development [70]. Concentrations of amino acids and fatty acids in blood and tissue samples have been reported to be higher among colorectal cancer patients compared to cancer-free individuals [68, 71]. But these studies were usually relatively small in sample size and limited in their ability to adjust for potential confounders or effect measure modifiers [72-74], such as age and sex [75].

Dietary intake has been linked to metabolic profiles of cancer patients [76, 77], potentially adding to the knowledge of the complex interaction between nutrition, cancer, and metabolism. Metabolites could add to the current understanding of the metabolic processes during disease development and progression [78-80]. Improving our understanding of the biological mechanisms underlying colorectal cancer development and progression, could be of interest from a clinical and nutritional perspective [81].

Metabolomics approaches

Metabolomics is defined as the quantitative and qualitative measurement of the metabolome in a biological system [66]. Two metabolomics approaches exist that aim to identify a broad range of molecules with diverse physiochemical properties [82, 83]. Briefly, untargeted metabolomics is highly sensitive, measures all molecules within a certain mass range and can also measure low abundant metabolites [84]. Sample analysis results in a relative quantification and untargeted metabolomics is generally used as a hypothesis-generating tool. It is a popular technique used to identify potential novel metabolites associated with the outcome of interest, but, analyzing untargeted data can be challenging and time-consuming [85]. In contrast, targeted metabolomics is a hypothesis-driven approach. It measures a set of predefined molecules and can (semi-) quantify the metabolite concentrations [82, 86]. It is, therefore, mostly used in studies with an interest in specific pathways in which the predefined metabolites are involved.

Knowledge gap

Metabolomics is increasingly used to gain insight in the biology of diseases, such as colorectal cancer [77, 79]. Although it is increasingly used, the sample sizes of previous studies are small and reproducibility of results between different studies is low. Reproducibility is important since analytical platforms (mainly of untargeted methods), study populations, used biospecimens, and study protocols vary a lot. So far, studies have also been limited in their ability to adjust for potential confounding and modifying effects, such as demographic, clinical and lifestyle factors, while this is essential to gain more knowledge regarding the underlying mechanisms of colorectal cancer development and prognosis.

Objectives

The main overarching objective of this thesis is **to improve our understanding of how metabolites are associated with the colorectal cancer continuum from a clinical and nutritional perspective**. To answer the overall objective of this thesis, specific aims were formulated:

1. To investigate which circulating metabolites are associated with colorectal cancer occurrence (**Chapter 2**) and colorectal cancer stage (**Chapter 3**).
2. To explore the association between dietary exposures and circulating metabolites in colorectal cancer patients (**Chapter 4**).
3. To assess the association between circulating folate and folic acid concentrations and recurrence and survival in colorectal cancer patients (**Chapter 5**).

International collaborations

To be able to address these research aims, a large sample size is needed. Therefore, the research aims above were embedded within two large international collaborations, namely the MetaboCCC and FOCUS consortium.

In 2012, the MetaboCCC consortium was initiated to investigate Metabolite profiles throughout the Continuum of Colorectal Carcinogenesis. Plasma samples of individuals free of colorectal tumors, patients with colorectal adenomatous polyps, and patients with colorectal cancer were collected among five research centers. The study populations included participants of the COLON study (Wageningen, the Netherlands) [56], CORSA study (Vienna, Austria), ColoCare study (Heidelberg, Germany) [58], NORCCAP study (Bergen, Norway), and EnCoRe study (Maastricht, the Netherlands) [57]. The final study population is shown in **Figure 2**. Targeted and untargeted metabolomics techniques were applied at IARC (Lyon, France).

In 2013, the partners within the MetaboCCC consortium initiated a second international consortium, namely the FOCUS consortium. In this FOCUS consortium, two additional sites of the ColoCare study (Utah and Seattle, US) joined (**Figure 2**). The aim of the FOCUS consortium was to explore metabolites related to Folate-dependent one-carbon

metabolism in relation to Colorectal cancer recurrence and Survival. Targeted metabolomics were performed at BEVITAL (Bergen, Norway).

Both IARC and BEVITAL fulfilled a central role in the respective consortia. Besides performing the metabolomics analysis on the blood samples, BEVITAL and IARC have an active role in the consortia as metabolomics experts and consult on appropriate data analysis tools and the interpretation of results.

Figure 2 Cohorts involved in the MetaboCCC and FOCUS consortia

Outline thesis

This thesis, aiming to better understand the association between (nutritional) metabolites along the colorectal cancer continuum, is divided in several chapters. To identify metabolites associated with colorectal cancer occurrence, plasma samples of healthy individuals and stage I-IV colorectal cancer patients are compared using an untargeted metabolomics approach (**Chapter 2**). Next, **Chapter 3** focusses on the association between plasma metabolites and individual colorectal cancer stages using targeted metabolomics. **Chapter 4** shows the associations between dietary exposures and plasma metabolites in colorectal cancer patients, using a targeted metabolomics approach. More specifically, the associations between two diet quality indexes and three dietary patterns, constructed using the dietary intake of the investigated study population, and plasma metabolites are tested. Circulating concentrations of folate and folic acid are assessed in relation to colorectal cancer recurrence and survival in **Chapter 5**.

In the final **Chapter 6**, the main findings of the studies are summarized and discussed. The presented results in this thesis are put into perspective, methodological and biological considerations, and consortia of the future are discussed. The general discussion also reviews encountered challenges and potential future directions for metabolomics research in the field of nutrition and cancer.

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*A clinical
perspective*

A large, white, stylized number '2' is centered on a dark, textured, and irregular shape that resembles a splash or a map of land. The shape is composed of various shades of gray and black, with a rough, splattered edge. The entire composition is set against a light gray background.

2

Chapter 2

Plasma metabolites and colorectal cancer

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Abstract

Colorectal cancer is known to arise from multiple tumorigenic pathways; however, the underlying mechanisms remain not completely understood. Metabolomics is becoming an increasingly popular tool in assessing biological processes. Previous metabolomics research focusing on colorectal cancer is limited by sample size and did not replicate findings in independent study populations to verify robustness of reported findings. Here, we performed a ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) screening on EDTA plasma from 268 colorectal cancer patients and 353 controls using independent discovery and replication sets from two European cohorts (ColoCare Study: n=180 patients / n=153 controls; the Colorectal Cancer Study of Austria (CORSA) n=88 patients / n=200 controls), aiming to identify circulating plasma metabolites associated with colorectal cancer and to improve knowledge regarding colorectal cancer aetiology. Multiple logistic regression models were used to test the association between disease state and metabolic features. Statistically significant associated features in the discovery set were taken forward and tested in the replication set to assure robustness of our findings. All models were adjusted for sex, age, BMI and smoking status and corrected for multiple testing using False Discovery Rate. Demographic and clinical data were abstracted from questionnaires and medical records.

A total of 691 metabolic features significantly discriminated between colorectal cancer patients and controls in the discovery stage, of which 97 features remained for replication. Metabolite identification yielded 28 annotated metabolites including: taurine, hypoxanthine, valine, leucine, bilirubin, 1-methylnicotinamide, and several glycerophospholipids. Our study provides novel insights regarding metabolic changes associated with colorectal cancer.

Keywords: UHPLC-QTOF-MS, colorectal cancer, discovery-replication approach, metabolomics

Introduction

Colorectal cancer is a major public health concern worldwide, with 1.4 million new cases and an estimated 700,000 deaths annually [1]. Colorectal cancer is characterized by a distinct metabolic phenotype and changes in key metabolic pathways such as glycolysis or the tricarboxylic acid (TCA) cycle [2, 3]. Yet, underlying mechanisms involved in colorectal carcinogenesis are still unclear [4].

Metabolomics is a powerful approach to unravel metabolic changes associated with disease and is gaining momentum in the field of cancer epidemiology [5-7]. Compared to other ‘-omics’ techniques, metabolomics is more closely related to a measured clinical phenotype and is increasingly applied as the method of choice to screen for potential metabolites associated with disease status [8, 9]. Moreover, metabolomics can help to understand the underlying aetiology of cancer development [10].

Differences in metabolic profiles have been reported between colorectal cancer patients and colorectal cancer-free individuals using nuclear magnetic resonance techniques [11], gas chromatography [12-15], and liquid chromatography-mass spectrometry methods [16].

Various amino acids, such as aspartic acid, have been shown to be more abundant in cases in different, relatively small, studies, including a study by Nishiumi and colleagues comparing serum metabolite levels of 60 colorectal cancer patients and 60 healthy volunteers using gas-chromatography time-of-flight (TOF) mass spectrometry [13]. Similarly, a study by Denkert *et al.* examined metabolic profiles in colon tissue and normal mucosa samples of 27 colorectal cancer patients and 18 colorectal cancer-free individuals [15]. In addition to amino acids, serum taurine was shown to be more abundant among colorectal cancer patients compared to colorectal cancer-free individuals. Another study among 101 newly diagnosed colorectal cancer patients reported a clear difference between serum glutamine, fatty acids, and the urea and TCA cycle metabolites compared to 102 colorectal cancer-free controls [17].

The majority of these previous studies have been limited by sample size and did not perform replication of their findings in independent study populations. As metabolomics studies often identified a wide range of metabolites due to the variety of analytical platforms, clinical protocols, and sample handling procedures used, leveraging an independent population for replication using the same platform and similar protocols is essential to ensure

robustness of findings. To date, only few studies have used a discovery-replication design to reproduce results in independent study populations [16, 18, 19]. Two of these studies investigated metabolic differences between colorectal cancer patients and apparently healthy individuals [16, 18]; a third study evaluated metabolomic differences between matched tumour and healthy colon tissue samples from colorectal cancer patients [19]. In addition, a very recent study investigating metabolic profiles in adenomas, colorectal cancer cases and controls conducted analysis in two datasets utilizing different metabolomic approaches, but with both sample sets deriving from the same hospital and cohort [20].

To complement current research, we utilized a powerful combination of untargeted metabolomics analysis, able to reveal (novel) metabolites, a rigorous discovery-replication design, leveraging samples deriving from two independent study populations, as well as relatively large sample sizes to obtain sufficient statistical power. The overall purpose of our study was to discover, and replicate plasma metabolites associated with colorectal cancer to improve knowledge regarding potential disease aetiology.

Methods

Study populations

We utilized data from two cohort studies embedded in the MetaboCCC Consortium, a consortium of four independent European cohorts to investigate metabolic profiles across the continuum of colorectal carcinogenesis: (1) the Heidelberg site of the international ColoCare Study (ClinicalTrials.gov Identifier: NCT02328677) and (2) the Colorectal Cancer Study of Austria (CORSA). The CORSA and ColoCare studies were selected given the availability of samples from colorectal cancer patients as well as controls. EDTA plasma samples from 621 participants were analysed, consisting of 268 patients with newly diagnosed colorectal cancer and 353 controls. We applied independent discovery (ColoCare Study: n=180 patients / n=153 controls) and replication (CORSA Study: n=88 patients / n=200 controls) sets using an identical metabolomics platform (**Supplementary Figure S1**).

The ColoCare Study, in Heidelberg initiated in 2010, is an ongoing, international, multi-centre prospective study including women and men newly diagnosed with primary colorectal cancer. Patients are recruited at the University Hospital of Heidelberg and the National Center for Tumor Diseases in Heidelberg, Germany. Participants provided consent prior to tumour resection if they met the following inclusion criteria: newly diagnosed

colorectal cancer (both colon (ICD-10 C18) and rectal or recto-sigmoidal cancer (ICD-10 C19/C20)), any stage of the disease, 18+ years at the time of diagnosis, and German-speaking. EDTA blood samples from colorectal cancer patients were collected prior to surgery. Control participants were enrolled in the PRAEVENT Study, a population-based study subjected to similar protocols and procedures, conducted at the National Center for Tumor Diseases in Heidelberg, Germany. All participants consented to take part in this study and EDTA blood samples were collected during a visit at the National Center for Tumor Diseases at recruitment (usually the same day after the consent dialogue and after signing the informed consent form).

In the ongoing CORSA Study participants are recruited in cooperation with the province-wide screening project “Burgenland Prevention Trial of Colorectal Disease with Immunological Testing” (B-PREDICT), since 2003. All inhabitants of the Austrian province Burgenland aged between 40 and 80 years are invited annually to participate in faecal occult blood testing. Positive faecal occult blood tested individuals are subsequently offered a complete colonoscopy, and EDTA blood samples are collected prior to examination. Additional colorectal cancer patients are recruited at the General Hospital of Vienna (Department of Surgery), and at three additional hospitals in Vienna. All colorectal cancer patients included in the CORSA Study are individuals with histologically confirmed, sporadic colorectal cancer. CORSA controls are individuals who received a complete colonoscopy within the B-PREDICT screening but exhibited no pathological findings of disease.

All colorectal cancer samples selected for inclusion into the presented study were collected prior to any clinical treatment, including surgery or neo-adjuvant therapy, and did not have a prior history of cancer. Controls included in the study can be considered as ‘cancer-free’; having no prior history of cancer. Patients and controls were 95% of Caucasian origin, recruited within the last fifteen years and selected to be matching according to their recruitment time point. Clinical data, including tumour location, staging, and treatment history were abstracted from medical records. Demographic characteristics (e.g. age, weight, height, and smoking status) were assessed by study-specific questionnaires. All clinical and demographic data were harmonized across all cohorts.

Sample collection and analysis

In both cohorts, non-fasted EDTA blood samples were collected and processed within four hours, according to identical processing protocols, and stored at -80°C. Samples at each

respective study site were shipped on dry ice to the International Agency for Research on Cancer (IARC) in Lyon, France for analysis. Samples were analysed with a ultra-high performance liquid chromatography- quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) system (Agilent Technologies) consisting of a 1290 Binary LC system, a Jet Stream electrospray ionization (ESI) source, and a 6550 QTOF mass spectrometer. Samples from each study centre were analysed in cohort-specific batches, which consisted of five and six 96-well plates for CORSA and ColoCare, respectively.

A detailed overview of the sample preparation and a complete description of sample analysis by UHPLC-QTOF-MS, pre-processing of metabolomics data can be found in **Supplemental File S1**. A summary of the data processing workflow is shown in **Supplementary Figure S1**.

Data Analysis

Features with missing values in >50% of either colorectal cancer patient or control samples in both populations were excluded from analysis. The remaining maximum 50% of missing values were not imputed according to the recommendations of Di Guida *et al.* [21]. Blank adjustment was applied for the ColoCare and CORSA samples separately; features that had a minimum relative mean intensity below the relative mean intensity of blank samples were removed. “Features” were defined as chromatographic peaks formed by specific ions, while “compounds” or “metabolites” referred to a confirmed molecule that can consist of one or more features (adducts, clusters and fragments).

Feature intensities were log transformed using the natural logarithm prior to statistical analysis, to prevent heteroscedasticity [21, 22]. Demographic and clinical characteristics are presented as medians with the interquartile range (IQR), or as numbers with corresponding percentages. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²). BMI status was categorized based on the recommendations from the World Health Organization (WHO): underweight (<18.5 kg/ m²), normal weight (18.5 – 24.9 kg/m²), overweight (25.0 – 29.9 kg/m²) and obese (≥30.0 kg/m²). Smoking status was categorized as current, former, and never.

Discovery stage

The discovery analysis was conducted in ColoCare samples. Log standardized odds ratios (OR.std) and 95% confidence intervals (CIs) were calculated using multiple logistic

regression models with disease state as dependent variable to test the association with feature intensities. The OR.std represents the change in colorectal cancer occurrence when there is a one standard deviation (SD) change in metabolite intensity, allowing comparison of effect sizes between different features. Since odds ratios were standardized, the SD of the controls were used to calculate the OR.std. Sex, age, BMI (continuous), and smoking status were included as covariates in the final model. Features that showed significant differences between colorectal cancer patients and controls after correction for multiple testing, using False Discovery Rate (FDR) correction, in the discovery stage were carried forward to the replication stage. *A priori*, an FDR p-value <0.05 was considered statistically significant.

Replication stage

The replication stage was conducted in CORSA Study samples. Significant features (FDR $p < 0.05$) from the discovery stage were analysed in the replication stage using the same modelling approach as in the discovery stage. Features were tested if they point in the same direction as the corresponding effects in the discovery stage (one-sided testing). Analyses were checked for any influence by analytical batch, but no marked effect could be identified in both stages. Features with significant test results were selected for identification using authentic chemical standards at IARC. A detailed overview of metabolite identification is explained in **Supplemental Table S1**. When more than one mass spectrometry feature corresponded with a metabolite, the feature with the highest intensity was selected and presented in the manuscript (**Supplementary Table S2**).

Spearman correlation analysis was used to identify metabolite-metabolite correlations among all identified metabolites and to understand the intra-relation of metabolites. Spearman correlation coefficients were calculated for all pairs of annotated features for samples from the discovery and replication set to account for deviations from linearity. All statistical analyses were performed in R, version 3.3.3 [23].

Results

Participant characteristics

Characteristics of the study population are summarized in **Table 1**. The ColoCare cohort consisted of 63% men in the colorectal cancer group and 38% men in the control group. In addition, ColoCare controls had on average less participants classified as

overweight compared to the colorectal cancer patients. The CORSA cohort consisted of 68% men in the colorectal cancer group and 65% men in the controls group. Control patients from the CORSA cohort had on average slightly more participants categorized as overweight compared to colorectal cancer patients.

In both cohorts control groups consisted of more participants categorized as never smokers than compared to the colorectal cancer patients. In general, the distributions of covariates were relatively comparable between the discovery and replication cohorts. Controls from the ColoCare cohort were 13 years younger than controls from the CORSA cohort. The majority of participants have a BMI classified as overweight, except for the controls from ColoCare.

Metabolic profiles discriminating between colorectal cancer patients and controls

Metabolomics analysis yielded 10,015 mass spectrometry features, defined as a chromatographic peak formed by specific ions that were identified across all study samples. After data pre-processing, 1,156 and 1,148 features were carried forward for ColoCare and CORSA samples, respectively.

Next, 691 out of 1,156 features were found to be statistically significantly associated with disease state (discovery stage) after FDR correction and adjustment for age, sex, BMI, and smoking status. The 691 significant features were subsequently analysed in the replication dataset, i.e. the CORSA Study samples. Of these features, 97 differed between CORSA patients and controls.

The 97 replicated discriminating mass spectrometry features corresponded to 28 metabolites, defined as a confirmed molecule that can consist of one or more features (adducts, clusters and fragments) (**Supplementary Table S2**). Six metabolites (taurine, hypoxanthine, valine, leucine, bilirubin, and 1-methylnicotinamide) were identified using authentic standards resulting in a level 1 identification according to the Metabolomics Standards Initiative (MSI), nine compounds (seven lysophosphatidylcholines (LysoPCs) and two lysophosphatidylethanolamines (LysoPEs)) reached MSI level 2 identification, and 14 compounds could not be identified (unknown metabolites, MSI level 4). The intensity of these 15 identified metabolites exhibited significant differences between colorectal cancer patients and controls in both the discovery and replication set (**Figure 1**). Taurine, hypoxanthine, LysoPE (20:4), and LysoPE (22:6) showed higher relative mean intensity values in the colorectal cancer group compared with controls, representing a OR.std higher than one (**Table**

2). Valine, leucine, bilirubin, 1-methylnicotinamide, and seven LysoPCs (LysoPC(15:0), LysoPC (16:0), LysoPC(16:0) isomer, LysoPC(P-16:0), LysoPC(16:1), LysoPC(17:0), LysoPC(18:0)) showed higher relative mean intensity values in the control group compared to the colorectal cancer patient group, indicating an OR.std lower than one (**Table 3**).

Correlation analysis

Spearman correlation analysis was used to identify potential metabolite-metabolite correlations among all identified metabolites (**Figure 2**). Correlation patterns demonstrated similar results across the discovery (**Figure 2A**) and replication stage (**Figure 2B**). For both stages, all LysoPCs were positively correlated (Spearman correlation coefficient range [r_s]: 0.40 - 0.91) but showed only a weak correlation to LysoPE (22:6) and LysoPE (20:4). Valine and leucine were highly correlated (discovery stage r_s : 0.73, replication stage r_s : 0.78). In addition, the majority of replicated compounds annotated as unknown (n=13) were correlated with each other but showed only weak correlations with the other annotated compounds. Spearman correlation coefficients are shown in **Supplementary Table S3**.

Table 1 Demographics and clinical characteristics of colorectal cancer patients and controls¹

	Discovery stage: ColoCare Study		Replication stage: CORSA	
	CRC patients	Controls	CRC patients	Controls
Number of participants	180	153	88	200
Sex ^{‡†}				
Male n(%)	114 (63.3)	59 (38.6)	60 (68.2)	130 (65.0)
Age ^{‡†}				
Median (IQR)	66.0 (58.0-73.0)	51.0 (42.0-63.0)	70.0 (60.0-76.0)	64.0 (57.0-74.0)
Body mass index ^{2,‡†} (kg/m ²)	26.4 (24.1-29.2)	23.5 (22.0-26.7)	26.1 (23.8-29.4)	27.0 (24.9-30.1)
	2 (1.1)	19 (12.4)	0 (0)	2 (1.0)
Underweight, <18.5 n(%)	59 (32.8)	3 (2.0)	26 (29.5)	47 (23.5)
Normal weight, 18.5-24.9 n(%)	82 (45.6)	91 (59.5)	38 (43.2)	94 (47.0)
Overweight, 25-29.9 n(%)	37 (20.6)	35 (22.9)	15 (17.0)	50 (25.0)
Obese, ≥30 n(%)	30 (16.6)	25 (16.3)	20 (22.7)	25 (12.5)
Smoking status* n(%)				
Current	77 (42.8)	47 (30.7)	30 (34.2)	64 (32.0)
Former	61 (33.9)	75 (49.1)	35 (39.7)	104 (52.0)
Never	12 (6.7)	6 (3.9)	3 (3.4)	7 (3.5)
Unknown	7 (3.9)	-	0 (0)	-
Stage ^{3,†} n(%)				
0	34 (18.9)	-	30 (34.1)	-
I	66 (36.7)	-	17 (19.3)	-
II	47 (26.1)	-	18 (20.5)	-
III	25 (13.9)	-	12 (13.6)	-
IV	1 (0.5)	-	3 (3.4)	-
Unspecified	0 (0)	-	8 (9.1)	-
Unknown	53 (29.4)	-	21 (31.8)	-
Colon – distal	57 (31.7)	-	33 (38.4)	-
Colon – proximal	70 (38.9)	-	34 (39.8)	-
Rectum				

¹Controls are defined as individuals not diagnosed with any colorectal malignancy; ²Missing BMI for n= 5, 9 and 7 for ColoCare controls, CORSA CRC patients and controls, respectively; ³TNM for colorectal cancer with neo-adjuvant therapy and colon cancers without surgery (28.7%), else pTNM was used (71.3%); ⁴Distal colon: sigmoid colon, descending colon, splenic flexure; Proximal colon: transverse colon, hepatic flexure, ascending colon, cecum, appendix; Rectum: rectum, rectosigmoid junction; *p-value<0.05 between ColoCare CRC and controls; ‡p-value<0.05 between CORSA CRC and controls; †p-value<0.05 between CRC; ‡p-value<0.05 between controls

Figure 1 Box plots of the 15 annotated metabolites differentiating colorectal cancer patients (CRC) and controls for the discovery and replication set. The boxplot presents the minimum, first quartile, median, third quartile and maximum log transformed relative intensity values and potential outliers of taurine, hypoxanthine, valine, leucine, bilirubin, 1-methylnicotinamide (MNA), LysoPC (15:0), LysoPC (16:0), LysoPC (16:0) isomer, LysoPC (P-16:0), LysoPC (16:1), LysoPC (17:0), LysoPC (18:0), LysoPE (20:4) and LysoPE (22:6), respectively.

Table 2 List of replicated metabolites **positively** associated with colorectal cancer¹

Compound Name	ID level ²	Discovery stage: ColoCare Study				Replication stage: CORSA			
		CRC Mean ± SD ³	Controls Mean ± SD ³	OR.std ⁴ [95% CI]	p value ⁵	CRC Mean ± SD ³	Controls Mean ± SD ³	OR.std ⁴ [95% CI]	p value ⁵
<i>Taurine</i>	1	12.53 ± 0.28	12.41 ± 0.24	1.44 [1.13; 1.87]	7.2E-03	12.96 ± 0.49	12.66 ± 0.34	1.95 [1.53; 2.51]	4.2E-06
<i>Hypoxanthine</i>	1	13.18 ± 1.32	11.73 ± 0.91	3.07 [2.25; 4.45]	9.1E-17	12.00 ± 0.53	11.79 ± 0.49	1.43 [1.09; 1.92]	3.6E-02
<i>LysoPE(20:4)</i>	2	12.64 ± 0.30	12.50 ± 0.26	1.37 [1.08; 1.76]	2.0E-02	12.66 ± 0.32	12.51 ± 0.32	1.51 [1.15; 2.01]	1.4E-02
<i>LysoPE(22:6)</i>	2	12.48 ± 0.40	12.21 ± 0.29	1.80 [1.42; 2.31]	1.5E-06	12.41 ± 0.34	12.28 ± 0.36	1.60 [1.20; 2.17]	7.8E-03
<i>171.0292@2.8578448</i>	4	10.32 ± 0.52	10.04 ± 0.40	1.61 [1.26; 2.08]	3.4E-04	10.2 ± 0.54	9.81 ± 0.41	2.04 [1.52; 2.81]	3.5E-05
<i>279.1473@2.7668757</i>	4	11.08 ± 0.83	10.59 ± 0.66	1.38 [1.09; 1.77]	1.5E-02	10.64 ± 0.84	9.97 ± 0.66	2.20 [1.62; 3.08]	1.0E-05
<i>428.3615@7.4910417</i>	4	9.61 ± 0.34	9.49 ± 0.26	1.49 [1.07; 2.15]	3.3E-02	9.56 ± 0.25	9.45 ± 0.18	1.59 [1.14; 2.26]	2.8E-02

¹Controls are defined as individuals not diagnosed with any colorectal malignancy; ²According to MSI; ³Log transformed relative intensity values; ⁴OR.std: standardized Odds Ratio, represents the relative change in colorectal cancer (CRC) risk when there is a one standard deviation (SD) change in metabolite intensity. OR.std is based on the SD of the controls; ⁵p-value: FDR-corrected p-value

Table 3 List of replicated metabolites **inversely** associated with colorectal cancer¹

Compound Name	ID level ²	Discovery stage: ColoCare Study				Replication stage: CORSA			
		CRC	Controls	OR.std ⁴	p value ⁵	CRC	Controls	OR.std ⁴	p value ⁵
		Mean ± SD ³	Mean ± SD ³	[95% CI]		Mean ± SD ³	Mean ± SD ³	[95% CI]	
<i>Leucine</i>	1	13.98 ± 0.49	14.07 ± 0.31	0.69 [0.53; 0.87]	4.4E-03	13.86 ± 0.32	13.93 ± 0.28	0.72 [0.55; 0.93]	4.7E-02
<i>1-methylnicotinamide</i>	1	11.18 ± 0.68	11.33 ± 0.60	0.69 [0.53; 0.88]	6.5E-03	11.11 ± 0.45	11.36 ± 0.56	0.62 [0.46; 0.83]	6.8E-03
<i>Valine</i>	1	10.36 ± 0.33	10.43 ± 0.33	0.74 [0.56; 0.96]	4.2E-02	10.18 ± 0.35	10.27 ± 0.27	0.69 [0.53; 0.89]	1.7E-02
<i>Bilirubin</i>	1	11.54 ± 0.59	11.74 ± 0.56	0.57 [0.43; 0.75]	1.5E-04	11.66 ± 0.64	11.92 ± 0.62	0.66 [0.49; 0.87]	1.5E-02
<i>LysoPC(16:1)</i>	2	10.90 ± 0.35	11.02 ± 0.27	0.60 [0.46; 0.77]	1.2E-04	10.31 ± 0.46	10.44 ± 0.36	0.73 [0.57; 0.93]	4.4E-02
<i>LysoPC(P-16:0)</i>	2	11.58 ± 0.42	11.68 ± 0.28	0.76 [0.61; 0.94]	2.0E-02	11.63 ± 0.42	11.81 ± 0.39	0.66 [0.50; 0.87]	1.4E-02
<i>LysoPC(15:0)</i>	2	11.90 ± 0.49	12.19 ± 0.36	0.53 [0.40; 0.67]	2.8E-07	11.69 ± 0.48	11.83 ± 0.43	0.71 [0.55; 0.92]	4.2E-02
<i>LysoPC(16:0)</i>	2	16.44 ± 0.38	16.52 ± 0.13	0.75 [0.61; 0.91]	5.7E-03	16.49 ± 0.26	16.61 ± 0.19	0.59 [0.46; 0.76]	3.3E-04
<i>LysoPC(16:0) isomer</i>	2	14.28 ± 0.45	14.44 ± 0.21	0.71 [0.55; 0.90]	8.3E-03	14.42 ± 0.38	14.59 ± 0.30	0.58 [0.42; 0.78]	2.9E-03
<i>LysoPC(17:0)</i>	2	12.22 ± 0.47	12.47 ± 0.36	0.54 [0.41; 0.69]	8.9E-07	12.11 ± 0.47	12.27 ± 0.40	0.68 [0.52; 0.88]	1.7E-02
<i>LysoPC(18:0)</i>	2	14.84 ± 0.39	15.00 ± 0.25	0.63 [0.50; 0.78]	5.7E-05	15.00 ± 0.42	15.18 ± 0.34	0.63 [0.49; 0.81]	2.9E-03
<i>157.1107@0.8299292</i>	4	11.45 ± 0.65	11.44 ± 0.61	0.69 [0.52; 0.90]	1.3E-02	11.39 ± 0.68	11.52 ± 0.66	0.67 [0.49; 0.90]	3.2E-02
<i>181.1113@5.475981</i>	4	10.37 ± 0.51	10.75 ± 0.47	0.41 [0.30; 0.55]	5.4E-09	10.88 ± 0.42	10.95 ± 0.33	0.72 [0.55; 0.93]	4.2E-02
<i>190.0061@0.83498704</i>	4	10.93 ± 0.32	11.04 ± 0.24	0.61 [0.46; 0.79]	3.3E-04	11.07 ± 0.39	11.21 ± 0.32	0.66 [0.51; 0.84]	6.1E-03
<i>247.0286@3.3104632</i>	4	12.88 ± 0.43	13.14 ± 0.31	0.43 [0.31; 0.57]	2.6E-09	12.99 ± 0.40	13.13 ± 0.32	0.66 [0.50; 0.86]	1.2E-02
<i>445.8809@0.56495804</i>	4	13.86 ± 0.36	13.96 ± 0.11	0.76 [0.64; 0.90]	3.0E-03	13.79 ± 0.31	13.87 ± 0.16	0.77 [0.61; 0.94]	4.2E-02
<i>452.8025@7.3067145</i>	4	9.77 ± 0.26	9.85 ± 0.28	0.72 [0.54; 0.96]	4.4E-02	9.88 ± 0.23	9.97 ± 0.21	0.65 [0.50; 0.86]	1.1E-02
<i>535.2997@7.0497603</i>	4	11.01 ± 0.41	11.16 ± 0.35	0.65 [0.49; 0.85]	3.4E-03	11.27 ± 0.38	11.37 ± 0.33	0.72 [0.55; 0.93]	4.4E-02
<i>545.3467@7.2500143</i>	4	13.39 ± 0.53	13.50 ± 0.31	0.79 [0.63; 0.98]	5.0E-02	13.78 ± 0.46	14.00 ± 0.39	0.61 [0.47; 0.78]	1.1E-03
<i>610.9343@7.191048</i>	4	11.78 ± 0.43	11.94 ± 0.34	0.63 [0.49; 0.80]	4.6E-04	11.34 ± 0.40	11.51 ± 0.33	0.63 [0.48; 0.82]	3.9E-03
<i>638.9663@7.4319897</i>	4	10.85 ± 0.33	10.97 ± 0.28	0.68 [0.53; 0.87]	4.7E-03	10.98 ± 0.29	11.13 ± 0.24	0.58 [0.44; 0.76]	4.5E-04

¹Controls are defined as individuals not diagnosed with any colorectal malignancy; ²According to MSI; ³Log transformed relative intensity values; ⁴OR.std: standardized Odds Ratio, represents the relative change in colorectal cancer (CRC) risk when there is a one standard deviation (SD) change in metabolite intensity. OR.std is based on the SD of the controls; ⁵p-value: FDR-corrected p-value

A

B

Figure 2 Metabolite-metabolite correlation analysis of replicated metabolites. Positive correlations are highlighted in blue, negative correlations are highlighted in red. Unknown compounds are indicated as monoisotopic mass@retention time. Metabolites are ordered by hierarchical clustering. (A) Spearman correlation analysis plot of the discovery dataset. (B) Spearman correlation analysis plot of the replication dataset.

Discussion

In this study, we identified plasma metabolites that are associated with colorectal cancer and which were replicated in an independent study population. We found 28 metabolites associated with disease state in two independent study cohorts, the ColoCare and CORSA studies. In total, 15 out of 28 metabolites could be identified. Taurine, hypoxanthine, valine, leucine, LysoPCs, and LysoPEs have been reported to be linked with colorectal cancer in previous metabolomics studies. All LysoPCs were positively correlated, valine and leucine were highly correlated, and the majority of unidentified metabolites were correlated with each other. Except for valine and leucine, the identified metabolites were only slightly or not correlated with each other.

Taurine was previously shown to be increased in serum of 60 colorectal cancer patients compared to 60 apparently healthy individuals [13] and in tumour tissue of 16 colorectal cancer patients [24]; which is in agreement with our findings. Recent studies have suggested taurine as a microbiota-associated metabolite playing a mediating role in microbiome-host interactions [25, 26]. Given the knowledge that gut microbiota differ between colorectal cancer patients and healthy individuals, and that microbial composition is linked to colorectal cancer risk [27], taurine presents a promising candidate for further investigation.

Hypoxanthine has been previously reported to be increased in tumour tissue of colorectal cancer patients compared to normal tissue of healthy individuals [15]. In contrast, a recent study, published by Long *et al.* reported decreased levels of hypoxanthine in colorectal cancer and polyps compared to controls [20]. Like taurine [5], hypoxanthine is an antioxidant and increased levels reported in the current study may be the result of increased oxidative stress [28], which is recognized as an important process in carcinogenesis, including colorectal cancer [29, 30]. Inconsistent findings in hypoxanthine levels may be due to the type of specimen analysed, or lack of statistical power because of lower sample numbers included. Furthermore, a possible reason for the inconsistent hypoxanthine levels may be caused by red blood cell haemolysis during the preparation of serum samples utilized in the Long study in contrast to plasma used in the present analysis [31].

With respect to branched-chain amino acids (BCAAs), we observed that valine was reduced among colorectal cancer patients compared to controls. This result is consistent with two prior studies; Ma and colleagues compared serum of 30 colorectal cancer patients to 30

colorectal cancer-free controls [12], and Farshidfar *et al.* investigated metabolomic signatures in colorectal cancer serum of stage I-IV patients [14]. Comparable to valine, decreased plasma levels of leucine were also reported in our colorectal cancer patients compared to controls. Decreased blood levels of BCAAs could reflect increased requirement for amino acids due to the high protein turnover in the malignant setting [15, 19, 32].

Moreover, seven LysoPCs were detected at lower levels among colorectal cancer patients compared to controls. LysoPC (16:0) and LysoPC (18:0) were reported before to be lower in the plasma of colorectal cancer patients versus control individuals [33, 34]. There seems to be a general trend of lower levels of LysoPCs among colorectal cancer patients in existing studies [17, 33, 35], which is in line with the findings reported in our study. This pattern might reflect an increased degradation rate of LysoPCs as a result of the accelerated cell proliferation rate of cancerous cells [36]. It has been suggested that decreased levels of LysoPCs could result from weight loss and possibly inflammatory processes related to cancer [37, 38]. While the majority of our study participants were classified as overweight, we did not have data on changes in body weight among patients prior to a colorectal cancer diagnosis.

LysoPE (20:4) and LysoPE (22:6) were increased in colorectal cancer patients compared to controls. LysoPEs belong to the group of signalling lipids and are constituents of cell membranes. Recently, serum LysoPEs were found to be elevated among breast cancer patients [39]. However, knowledge is limited regarding the role of LysoPEs in healthy and diseased individuals.

We also identified a notable decrease in MNA, an inactive metabolite of nicotinamide [40], among colorectal cancer patients compared to controls. MNA has been reported *in vivo* to be involved in the COX-2/PGI₂ pathway [40], which plays a major role in inflammation and colorectal carcinogenesis [41, 42]. In addition, this is the first metabolomics study to report lower plasma bilirubin levels in colorectal cancer patients compared to controls. Previously, a European study analysing genomic alterations in promoter variants involved in bilirubin homeostasis, and another study investigating serum bilirubin levels in a large U.S. population have proposed a protective effect of bilirubin against colorectal carcinogenesis [43, 44]; our metabolomics findings carefully support this hypothesis. The underlying mechanisms of the relationship between bilirubin and colorectal cancer remain unclear.

Untargeted metabolomics is an elegant approach for the discovery of metabolites associated with cancer. However, one may wonder whether the seemingly small differences between colorectal cancer patients and controls are biologically relevant. It is important to keep in mind that findings presented are log transformed relative values. Consequently, reported results hint towards the direction of the association and quantification of the metabolites is needed to be able to interpret absolute differences. Our results for taurine, hypoxanthine, valine, leucine, bilirubin, and 1-methylnicotinamide suggest future research to investigate the underlying biological mechanism of these metabolites in relation to colorectal cancer.

A strength of the present study is the use of a discovery-replication design leveraging two independent, relatively large, patient cohorts, both including patients of Caucasian origin, from two different countries. In general, untargeted methods typically yield data with high amounts of noise and non-biological information [45]. This makes replication of untargeted metabolomics findings within ethnically homogenous cohorts extremely valuable, as it enables the exclusion of features that are not robustly associated with the case-control status.

A limitation of the current study is that due to recruitment procedures we tend to have more early stage colorectal cancer cases (stage I-II) compared to advanced metastatic patients (stage IV). This may indicate that our findings are mostly associated with early metabolic changes in colorectal carcinogenesis rather than with metastatic formation. Furthermore, findings are derived from cross-sectional data. Therefore, it is not possible to explore to which extent metabolites are causally related to cancer or cancer-related changes. Lastly, although our study was performed using a single stringent metabolomics approach across two independent populations, we acknowledge that metabolomics assays can be conducted using a variety of analytical platforms. As such, future studies should include multiple platforms to ensure the highest analytical coverage of the metabolome. Technical progress and the development of more comprehensive metabolite databases will also be needed to improve annotation of unknown compounds, including the unknown metabolites in the current study. Future targeted approaches, allowing the quantitative measurement of metabolites, would allow quantification of their absolute concentrations [46, 47].

In summary, this study provides new evidence of associations of colorectal cancer with plasma metabolites and confirms some evidence of previous findings.

The combination of an untargeted metabolomics approach, a rigorous discovery-replication design utilizing large sample sizes from independent cohorts, led to the identification and replication of 28 metabolites associated with colorectal cancer, including 15 metabolites that could be identified. These 15 identifiable metabolites should be carried forward as candidates for targeted analysis in prospective cohort studies, preferably derived from a colorectal cancer screening program, to verify their discriminating or potential predicting properties. Our study provides important leads for further studies focusing on metabolic differences between colorectal cancer-free individuals, and patients with different stages of colorectal cancer. Together, our findings emphasize the power of metabolomics as a strong molecular approach for gaining novel insights regarding metabolic changes associated with colorectal cancer.

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Ethical approval and consent to participate Written informed consent was obtained from all participants. The ColoCare Study has been approved by the ethics committee of the Medical Faculty at the University of Heidelberg. The CORSA Study was approved by the ethical review committee of the Medical University of Vienna (1160/2016), by the “Ethikkommission der Stadt Wien” (06-150-VK) and by the institutional review board “Ethikkommission Burgenland”. The study was also approved by the International Agency for Research on Cancer ethics committee.

Competing Interests Michael M. Bergman is founder of the biotechnology company Vacthera BioTech GmbH (Vienna, Austria) and received a consultant fee (<2000€) from Dr. Falk GmbH (Vienna, Austria). Cornelia M. Ulrich (Director of the Comprehensive Cancer Center at Huntsman Cancer Institute, Salt Lake City, USA) oversees research funded by several pharmaceutical companies but has not received funding directly herself.

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Supplementary materials

Supplemental File S1

Sample preparation and analysis by UHPLC-QTOF-MS

Samples were randomized across cohort-specific batches and prepared by mixing 20 μL of plasma with 200 μL of acetonitrile. After centrifugation (500 g, 4°C, 10 min), the supernatants were vacuum filtered into 1 ml polypropylene 96-well plates that were sealed and kept at 4°C in the liquid chromatography-mass spectrometry autosampler until analysis (Captiva ND 0.2 μm filter and collection plates, Agilent Technologies, Santa Clara, CA; EPS well plate seals, BioChromato, San Diego, CA). Quality control samples were prepared from a pool of study samples. One blank sample per well plate was prepared along the EDTA plasma samples in an identical manner, only leaving the plasma out of the process.

The auto sampler tray was kept at 4°C and 2 μL of the sample solution was injected on an ACQUITY UHPLC HSS T3 column (2.1 $\times$ 100 mm, 1.8 μm ; Waters, Milford, MA). Column temperature was maintained at 45°C with a mobile phase flow rate of 0.4 ml/min, consisting of ultrapure water and LC-MS grade methanol, both containing 0.05% (v/v) of formic acid. The gradient profile was as follows: 0–6 min: from 5% to 100% methanol, 6–10.5 min: 100% methanol, 10.5–13 min: 5% methanol.

The mass spectrometer was operated in positive ion mode using the following conditions: drying gas (nitrogen) temperature 175°C and flow 12 L/min, sheath gas temperature 350°C and flow 11 L/min, nebulizer pressure 45 psi, capillary voltage 3500 V, nozzle voltage 300 V, and fragmentor voltage 175 V. Data acquisition was performed using 2 GHz extended dynamic range mode across a mass range of 50–1000. Acquisition rate was 1.67 Hz in centroid mode. Continuous mass axis calibration was performed with two reference ions (m/z 121.050873 and m/z 922.009798). Data was acquired using MassHunter Acquisition B.05.01 (Agilent Technologies). The analytical run was initiated with priming injections of a quality control sample to achieve stable instrument response, followed by a blank and the study samples.

The processing of acquired metabolomics data was performed using Qualitative Analysis B.06.00, DA reprocessor, and Mass Profiler Professional 12.1 software (Agilent

Technologies). Recursive feature finding was employed to find compounds as singly charged ions $[M+H]^+$. The initial processing of the data was performed using Qualitative Analysis with the molecular feature extraction algorithm for small molecules. Thresholds for mass and chromatographic peak heights were 1500 and 10000 counts, respectively. Peak spacing tolerance for isotope peaks was $0.0025\ m/z$ plus 7 ppm, with the isotope model set to common organic molecules, and with single-ion features also allowed.

Pre-processing of metabolomics data

Features found in at least 2% of all samples were combined into a target list, using 0.08 min retention time and $15\ \text{ppm} + 2\ \text{mDa}$ mass windows for alignment. The recursive feature extraction was performed using a find by formula algorithm with match tolerances for the mass and retention time at $\pm 10\ \text{ppm}$ and $\pm 0.04\ \text{min}$. Ions were limited to $[M+H]^+$.

Alignment was performed with Mass Profiler Professional 12.1 by combining the four compound exchange (.cef) files obtained from the pre-processing of each dataset, using mass and retention time windows of $\pm 15\ \text{ppm}$ and $\pm 0.07\ \text{min}$. Successful alignment was facilitated by the high inter and intra-batch reproducibility of retention times, which was assessed by monitoring 13 preselected model compounds in quality control samples, for which retention time variability was less than 0.025 min across all the batches. Features with intensity values below 10,000 in all samples were removed from the analysis.

Metabolite Identification

Metabolite identification for UHPLC-QTOF-MS was performed to aim for level 1 identification according to The metabolomics standard initiative.[48] Features were clustered by retention time and intensity correlation to help in finding related ions (**Supplementary Table S1**), and m/z values were searched against the human metabolite database (HMDB 4.0) [49] using ions $[M+H]^+$, $[M+Na]^+$, $[M-H_2O+H]^+$, and $[M+2H]^+$, with 7 ppm molecular weight tolerance. Quality of the chromatographic peaks and spectra were inspected, and the plausibility of database candidates was assessed based on retention time, isotope pattern, adduct formation, and neutral losses. Identification was confirmed by analysing pure chemical standards and representative study samples, and by comparing the retention times and MS/MS spectra obtained. When standards were not available, the MS/MS spectra were compared against those in mzCloud [50] or Metlin [51]. The level of identification was determined as proposed by Sumner *et al.* [52]. Details of identification are provided in **Supplementary Table S1**.

Supplementary Figure S1 Workflow and study design. (A) The study population consisted of 268 colorectal cancer patients and 353 controls (individuals not diagnosed with any colorectal malignancy), including an independent discovery (ColoCare Study: n=180 patients/n=153 controls) and replication set (CORSa Study: n=88 patients/n=200 controls). (B) Study workflow including feature finding and data alignment (raw data processing), data processing, data analysis (discovery-replication setting) and identification of replicated features.

Supplementary Table S1 Identification of metabolites**General notes:**

- Chromatograms and spectra are from representative study samples and pure chemical standards when available. All chemical standards were from IROA Technologies' Mass Spectrometry Metabolite Library (MSMLS)
- Chromatograms and isotope patterns: Find Compounds by Formula searches in Agilent MassHunter Qualitative Analysis B.06.00 SP1
- Isotope patterns: Rectangles represent an isotope pattern calculated from the elemental composition (shown in the title of each spectra), bars inside the rectangles show the observed isotope peaks
- MS/MS spectra: Precursor is indicated with a blue dot above the ion. Collision energy is on top of the spectra, e.g. CID@20.0.

1-Methylnicotinamide

Taurine

Valine

Valine was found as $[M+Na]^+$ due to saturation of the principal ion $[M+H]^+$. MS/MS spectra acquired from $[M+H]^+$

Hypoxanthine

Leucine

LysoPC(16:1)

LysoPC(16:1) found as $[M+H+K]^{2+}$. MS/MS was acquired from $[M+H]^+$

LysoPC(15:0)

LysoPE(22:6)

LysoPE(20:4)

LysoPC(16:0) isomer

LysoPC(16:0)

LysoPC(P-16:0)

LysoPC(17:0)

LysoPC(18:0)

Bilirubin

Bilirubin typically exhibits high number of ions in electrospray (see isotope patterns below). MS/MS was acquired from [M+H]⁺

Supplementary Table S2 Metabolite identifications of the 97 replicated features

If applicable, the light gray features represent the most intense feature belonging to a compound.

cpd#	monoisotopic mass @retention time	Compound Name	Level according to the Metabolomics Standards Initiative
1	122.9206@0.56447005	Unknown	4
	205.8796@0.5669058		
	207.8776@0.56648636		
	273.8663@0.56767327		
	289.8411@0.5683634		
	341.8549@0.56762207		
	357.8274@0.56829727		
	409.8426@0.56771487		
	425.8154@0.5682527		
	445.8809@0.56495804		
	493.8012@0.56823456		
	513.8702@0.5653107		
	561.7904@0.5682394		
	565.8809@0.56176305		
	581.8576@0.56466657		
	623.8351@0.5579561		
	629.7794@0.56837577		
	649.8467@0.5632472		
	657.8285@0.55736333		
	691.8246@0.556467		
	697.7639@0.56842214		
	717.8281@0.5610313		
	765.7508@0.56839013		
	785.8171@0.5577929		
	853.8052@0.5547323		
	921.7903@0.55131626		
	937.7639@0.559643		

2	136.0636@0.58916837	1-methylnicotinamide	1
3	125.0146@0.6277308	Taurine	1
4	71.074@0.8027233 139.0611@0.80280536	Valine	1
5	157.1107@0.8299292	Unknown	4
6	190.0061@0.83498704	Unknown	4
7	136.0381@1.1621803 136.0384@0.85686004	Hypoxanthine	1
Note: 136.0384@0.85686004 most likely a split peak of hypoxanthine. This was confirmed in the Spearman correlation plot. Highly correlated with hypoxanthine and, therefore, considered to be a feature belonging to hypoxanthine.			
8	85.0889@1.4623561	Leucine	1
9	279.1473@2.7668757	Unknown	4
10	171.0292@2.8578448	Unknown	4
11	143.0166@3.3154876 200.0362@3.3102927 214.0152@3.31054 231.9886@3.3105862 232.0561@3.3086596 232.0574@3.310062 245.0312@3.3104632 246.0422@3.310548 247.0286@3.3104632 250.0351@3.310955 261.0059@3.3104744	Unknown	4

	264.0146@3.3119278 282.0348@3.3101916		
12	181.1113@5.475981	Unknown	4
13	266.1351@6.8201194	LysoPC(16:1)	2
14	481.3159@6.8777747	LysoPC(15:0)	2
15	525.2864@6.888085	LysoPE(22:6)	2
16	501.285@6.8980646 523.2694@6.8969865	LysoPE(20:4)	2
17	495.3333@6.9326496	LyosPC(16:0) isomer	2
18	523.2949@6.996259 523.7955@6.9962544 522.2939@6.9969144 522.7951@6.996832 762.478@6.997659 770.46@6.997441 761.976@6.99754 769.9581@6.9974766 517.3147@6.9961543 506.3191@6.9973693 495.3327@6.9956923 990.6655@6.9950514	LysoPC (16:0)	2
19	527.312@7.05021 527.8146@7.050606 535.2997@7.0497603	Unknown	4

20	479.3405@7.1102185	LysoPC(P-16:0)	2
21	509.3468@7.1235833	LysoPC(17:0)	2
22	602.9412@7.1884513 597.4188@7.187734 610.9343@7.191048 611.4359@7.191536	Unknown	4
23	103.0997@7.241595 523.364@7.2414613 534.8485@7.241308 534.3476@7.2410583 542.3386@7.2418456 542.8411@7.2418675 550.3265@7.2414513 550.8254@7.2414603	LysoPC(18:0)	2
24	545.3467@7.2500143	Unknown	4
25	452.8025@7.3067145	Unknown	4
26	639.4673@7.4324927 638.9663@7.4319897	Unknown	4
27	428.3615@7.4910417	Unknown	4
28	284.1159@7.9399796 314.1271@7.94182 580.232@7.9397755 612.2575@7.940997 644.2827@7.9412355	Bilirubin	1

Supplementary Table S3 Spearman correlation coefficients of the 28 metabolites in the discovery and replication stage

Discovery stage	190.0061 @0.83498	247.0286 @3.31046	Valine	Leucine	445.8809 @0.56495	l-methyl- nicotinamide	LysoPE(22 :6)	LysoPE(20 :4)	428.3615 @7.49104	279.1473 @2.76687	171.0292 @2.85784	610.9343 @7.19104	LysoPC(p- 16:0)	LysoPC(16 :0) isomer
190.0061@0.83498704	1													
247.0286@3.3104632	0.31	1												
Valine	0.18	0.56	1											
Leucine	0.02	0.66	0.73	1										
445.8809@0.56495804	0.21	0.06	0.26	0.27	1									
l-methylnicotinamide	0.01	0.15	0.18	0.18	0.23	1								
LysoPE(22:6)	-0.02	-0.11	-0.05	-0.04	0.10	-0.11	1							
LysoPE(20:4)	-0.06	0.02	-0.01	0.04	0.05	-0.11	0.53	1						
428.3615@7.4910417	-0.03	-0.05	0.10	0.01	0.02	-0.06	0.12	0.13	1					
279.1473@2.7668757	-0.09	-0.12	-0.05	-0.08	-0.09	-0.09	0.21	0.04	0.15	1				
171.0292@2.8578448	0.02	-0.18	-0.18	-0.20	-0.04	-0.14	0.22	0.01	0.12	0.58	1			
610.9343@7.191048	0.16	0.20	0.22	0.13	0.14	-0.05	0.01	0.12	0.00	-0.11	-0.10	1		
LysoPC(p-16:0)	0.13	0.18	0.16	0.12	0.10	-0.09	0.07	0.16	0.01	-0.02	-0.07	0.60	1	
LyosPC(16:0) isomer	0.19	0.32	0.27	0.28	0.06	-0.10	0.09	0.17	-0.02	0.00	-0.05	0.68	0.70	1
LysoPC (16:0)	0.16	0.31	0.27	0.25	0.07	-0.08	0.17	0.25	-0.03	-0.02	-0.07	0.66	0.77	0.90
LysoPC(18:0)	0.09	0.21	0.23	0.19	0.12	-0.10	0.04	0.16	0.04	-0.09	-0.13	0.75	0.79	0.83
545.3467@7.2500143	-0.09	0.10	0.10	0.20	0.16	-0.06	0.14	0.23	-0.01	-0.06	-0.12	0.61	0.68	0.71
LysoPC(15:0)	0.13	0.23	0.16	0.18	0.09	-0.13	-0.01	-0.04	-0.10	-0.04	-0.03	0.47	0.56	0.63
LysoPC(17:0)	0.12	0.21	0.15	0.18	0.13	-0.17	0.03	0.03	-0.09	-0.05	-0.07	0.60	0.70	0.68
LysoPC(16:1)	0.13	0.21	0.12	0.11	-0.01	-0.03	0.08	0.26	-0.08	-0.12	-0.10	0.34	0.44	0.63
535.2997@7.0497603	0.27	0.31	0.26	0.11	0.05	-0.07	0.03	0.14	-0.07	-0.07	-0.08	0.57	0.63	0.63
157.1107@0.8299292	-0.09	0.02	-0.01	0.07	0.07	0.07	0.03	-0.02	0.06	0.08	-0.02	0.15	0.16	0.10
181.1113@5.475981	0.15	0.27	0.13	0.14	0.10	0.12	-0.17	0.01	-0.08	-0.09	-0.17	0.26	0.22	0.27
Taurine	-0.11	-0.13	0.05	0.13	0.05	0.11	0.09	0.00	-0.06	0.11	0.05	0.00	0.05	0.11
Hypoxanthine	-0.26	-0.07	0.11	0.12	-0.17	-0.07	0.25	0.16	0.16	0.18	0.09	0.03	0.08	0.13
Bilirubin	-0.09	0.30	0.18	0.37	0.24	0.27	-0.05	0.04	0.04	-0.05	-0.14	0.11	0.21	0.17
452.8025@7.3067145	-0.18	0.09	0.03	0.20	0.02	-0.04	-0.06	0.00	-0.12	-0.09	-0.13	0.41	0.33	0.43
638.9663@7.4319897	-0.17	0.12	0.02	0.19	0.03	-0.09	0.01	0.15	-0.07	-0.11	-0.13	0.56	0.51	0.60

Replication stage

	190.0061 @0.83498	247.0286 @3.31046	Valine	Leucine	445.8809 @0.56495	1-methyl- nicotinamide	LysoPE(22 :6)	LysoPE(20 :4)	428.3615 @7.49104	279.1473 @2.76687	171.0292 @2.85784	610.9343 @7.19104	LysoPC(P- 16:0)	LysoPC(16 :0) isomer
190.0061@0.83498704	1	0.10	0.05	-0.06	0.21	-0.04	0.02	-0.04	-0.18	-0.19	-0.05	0.09	0.09	0.22
247.0286@3.3104632	0.10	1	0.56	0.69	0.27	0.27	0.07	0.04	-0.14	-0.24	-0.23	0.29	0.23	0.24
Valine	0.05	0.56	1	0.78	0.45	0.16	0.11	0.12	-0.02	-0.05	-0.11	0.43	0.33	0.33
Leucine	-0.06	0.69	0.78	1	0.46	0.15	0.08	0.16	-0.03	-0.04	-0.05	0.39	0.30	0.34
445.8809@0.56495804	0.21	0.27	0.45	0.46	1	0.11	0.27	0.36	-0.09	0.10	0.00	0.54	0.49	0.46
1-methylnicotinamide	-0.04	0.23	0.16	0.15	0.11	1	-0.02	-0.13	-0.07	-0.23	-0.24	0.12	0.10	0.06
LysoPE(22:6)	0.02	0.07	0.11	0.08	0.27	-0.02	1	0.58	0.07	0.16	0.08	0.23	0.21	0.22
LysoPE(20:4)	-0.04	0.04	0.12	0.16	0.36	-0.13	0.58	1	-0.03	0.22	0.13	0.38	0.35	0.34
428.3615@7.4910417	-0.18	-0.14	-0.02	-0.03	-0.09	-0.07	0.07	-0.03	1	0.28	0.28	-0.15	-0.21	-0.33
279.1473@2.7668757	-0.19	-0.24	-0.05	-0.04	0.10	-0.23	0.16	0.22	0.28	1	0.58	-0.03	-0.02	-0.03
171.0292@2.8578448	-0.05	-0.23	-0.11	-0.05	0.00	-0.24	0.08	0.13	0.28	0.58	1	-0.18	-0.14	-0.09
610.9343@7.191048	0.09	0.29	0.43	0.39	0.54	0.12	0.23	0.38	-0.15	-0.03	-0.18	1	0.79	0.82
LysoPC(P-16:0)	0.09	0.23	0.33	0.30	0.49	0.10	0.21	0.35	-0.21	-0.02	-0.14	0.79	1	0.80
LysoPC(16:0) isomer	0.22	0.24	0.33	0.34	0.46	0.06	0.22	0.34	-0.33	-0.03	-0.09	0.82	0.80	1
LysoPC(16:0)	0.23	0.35	0.43	0.40	0.53	0.09	0.28	0.36	-0.24	-0.08	-0.18	0.85	0.85	0.93
LysoPC(18:0)	0.14	0.25	0.40	0.35	0.52	0.09	0.20	0.36	-0.17	-0.03	-0.19	0.91	0.86	0.85
545.3467@7.2500143	0.08	0.27	0.33	0.34	0.38	0.16	0.24	0.33	-0.15	-0.19	-0.26	0.65	0.60	0.62
LysoPC(15:0)	0.17	0.16	0.31	0.24	0.40	0.16	0.28	0.22	-0.16	-0.02	-0.19	0.58	0.61	0.64
LysoPC(17:0)	0.19	0.18	0.33	0.28	0.46	0.13	0.29	0.29	-0.19	-0.02	-0.20	0.69	0.74	0.72
LysoPC(16:1)	0.15	0.12	0.24	0.15	0.37	0.06	0.32	0.40	-0.21	-0.07	-0.18	0.64	0.59	0.66
535.2997@7.0497603	0.21	0.34	0.38	0.36	0.52	0.11	0.31	0.47	-0.17	-0.05	-0.14	0.77	0.74	0.77
157.1107@0.8299292	0.00	0.05	0.15	0.23	0.20	0.07	0.15	0.25	-0.02	0.05	-0.06	0.21	0.16	0.14
181.1113@5.475981	-0.05	0.20	0.15	0.18	0.12	0.10	-0.08	0.11	-0.19	-0.06	-0.07	0.20	0.16	0.15
Taurine	-0.24	-0.08	0.00	-0.01	-0.02	-0.08	0.18	0.10	0.24	0.27	0.23	-0.10	-0.11	-0.16
Hypoxanthine	0.02	0.03	0.14	0.11	0.08	-0.11	0.13	0.16	0.12	-0.10	0.05	0.14	0.06	0.08
Bilirubin	-0.08	0.02	0.31	0.36	0.20	0.19	0.06	0.12	-0.21	-0.25	-0.25	0.33	0.36	0.35
452.8025@7.3067145	0.00	0.16	0.30	0.29	0.28	0.08	0.12	0.19	-0.14	-0.07	-0.20	0.78	0.62	0.71
638.9663@7.4319897	0.04	0.19	0.32	0.31	0.36	0.09	0.12	0.26	-0.17	-0.09	-0.26	0.80	0.71	0.75

Replication stage

Replication stage	LysoPC (16:0)	LysoPC(18 :0)	LysoPC(18 :0)	LysoPC(15 :0)	LysoPC(17 :0)	LysoPC(16 :1)	535.2997 @7.04976	157.1107 @0.82992	181.1113 @5.47598	Taurine	Hypoxanthine	Bilirubin	452.8025 @7.30671	638.9663 @7.43198
190.0061@0.83498704	0.23	0.14	0.08	0.17	0.19	0.15	0.21	0.00	-0.05	-0.24	0.02	-0.08	0.00	0.04
247.0286@3.3104632	0.35	0.25	0.27	0.16	0.35	0.18	0.34	0.05	0.20	-0.08	0.03	0.32	0.16	0.19
Valine	0.43	0.40	0.33	0.31	0.33	0.24	0.38	0.15	0.15	0.00	0.14	0.31	0.30	0.32
Leucine	0.40	0.35	0.34	0.24	0.28	0.15	0.36	0.23	0.18	-0.01	0.36	0.29	0.31	0.31
445.8809@0.56495804	0.53	0.52	0.38	0.40	0.46	0.46	0.52	0.20	0.12	-0.02	0.08	0.20	0.28	0.36
1-methylnicotinamide	0.09	0.09	0.16	0.16	0.13	0.06	0.11	0.07	0.10	-0.08	-0.11	0.19	0.08	0.09
LysoPE(22:6)	0.28	0.20	0.24	0.28	0.29	0.32	0.31	0.15	-0.08	0.18	0.13	0.06	0.12	0.12
LysoPE(20:4)	0.37	0.36	0.33	0.22	0.29	0.40	0.47	0.25	0.11	0.10	0.16	0.12	0.19	0.26
428.3615@7.4910417	-0.24	-0.17	-0.15	-0.16	-0.19	-0.21	-0.17	-0.02	-0.19	0.24	0.12	-0.21	-0.14	-0.17
279.1473@2.7668757	-0.08	-0.03	-0.19	-0.02	-0.02	-0.07	-0.05	0.05	-0.06	0.27	-0.10	-0.25	-0.07	-0.09
171.0292@2.8578448	-0.18	-0.19	-0.26	-0.19	-0.20	-0.18	-0.14	-0.06	-0.07	0.23	0.05	-0.25	-0.20	-0.26
610.9343@7.191048	0.85	0.91	0.65	0.58	0.69	0.64	0.77	0.21	0.20	-0.10	0.14	0.33	0.78	0.80
LysoPC(P-16:0)	0.85	0.86	0.60	0.61	0.74	0.59	0.74	0.16	0.16	-0.11	0.06	0.36	0.62	0.71
LysoPC(16:0) isomer	0.93	0.85	0.62	0.64	0.72	0.66	0.77	0.14	0.15	-0.16	0.08	0.35	0.71	0.75
LysoPC(16:0)	1	0.91	0.65	0.67	0.75	0.71	0.83	0.15	0.19	-0.15	0.12	0.32	0.67	0.73
LysoPC(18:0)	0.91	1	0.68	0.62	0.75	0.65	0.77	0.17	0.17	-0.12	0.11	0.28	0.72	0.81
545.3467@7.2500143	0.65	0.68	1	0.42	0.53	0.41	0.52	0.18	0.07	-0.07	0.18	0.31	0.61	0.65
LysoPC(15:0)	0.67	0.62	0.42	1	0.91	0.55	0.58	0.16	0.12	-0.11	0.04	0.27	0.42	0.51
LysoPC(17:0)	0.75	0.75	0.53	0.91	1	0.53	0.67	0.21	0.16	-0.15	0.00	0.28	0.50	0.61
LysoPC(16:1)	0.71	0.65	0.41	0.55	0.53	1	0.65	0.10	0.04	-0.10	0.16	0.27	0.50	0.56
535.2997@7.0497603	0.83	0.77	0.52	0.58	0.67	0.65	1	0.16	0.24	-0.10	0.09	0.30	0.50	0.56
157.1107@0.8299292	0.15	0.17	0.18	0.16	0.21	0.10	0.16	1	0.07	0.04	-0.03	0.17	0.12	0.14
181.1113@5.475981	0.19	0.17	0.07	0.12	0.16	0.04	0.24	0.07	1	0.02	0.05	0.11	0.10	0.12
Taurine	-0.15	-0.12	-0.07	-0.11	-0.15	-0.10	-0.10	0.04	0.02	1	0.21	-0.14	-0.13	-0.15
Hypoxanthine	0.12	0.11	0.18	0.04	0.00	0.16	0.09	-0.03	0.05	0.21	1	0.07	0.15	0.09
Bilirubin	0.32	0.28	0.31	0.27	0.28	0.27	0.31	0.17	0.11	-0.14	0.07	1	0.39	0.40
452.8025@7.3067145	0.67	0.72	0.61	0.42	0.50	0.50	0.50	0.12	0.10	-0.13	0.15	0.39	1	0.87
638.9663@7.4319897	0.73	0.81	0.65	0.51	0.61	0.56	0.56	0.14	0.12	-0.15	0.09	0.40	0.87	1

Chapter 3

Plasma metabolites and colorectal cancer stage

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Abstract

Colorectal cancer is the second most common cause of cancer-related death globally, with marked differences in prognosis by disease stage at diagnosis. We studied circulating metabolites in relation to disease stage to improve the understanding of metabolic pathways related to colorectal cancer progression. We investigated plasma concentrations of 130 metabolites among 744 stage I-IV colorectal cancer patients from ongoing cohort studies. Plasma samples, collected at diagnosis, were analysed with liquid chromatography-mass spectrometry using the Biocrates AbsoluteIDQ™ p180 kit. We assessed associations between metabolite concentrations and stage using multinomial and multivariable logistic regression models. Analyses were adjusted for potential confounders as well as multiple testing using false discovery rate (FDR) correction. Patients presented with 23%, 28%, 39% and 10% of stage I-IV disease, respectively. The concentrations of sphingomyelin C26:0 was lower in stage III patients compared to stage I patients ($p_{\text{FDR}} < 0.05$). Concentrations of sphingomyelin C18:0 and phosphatidylcholine (diacyl) C32:0 were statistically significantly higher, while citrulline, histidine, phosphatidylcholine (diacyl) C34:4, phosphatidylcholine (acyl-alkyl) C40:1 and lysophosphatidylcholines (acyl) C16:0 and C17:0 concentrations were lower in stage IV compared to stage I patients ($p_{\text{FDR}} < 0.05$). Our results suggest that metabolic pathways involving amongst others citrulline and histidine, implicated previously in colorectal cancer development, may also be linked to colorectal cancer progression.

Keywords: Colorectal cancer, disease stage, metabolomics, plasma metabolites, epidemiology

Introduction

For colorectal cancer, disease stage at diagnosis is one of the key determinants of prognosis and survival [1, 2]. To date, the mechanisms underlying cancer progression remain incompletely understood. In the current study, we aimed to study the association between circulating plasma metabolites at diagnosis and colorectal cancer stage.

Metabolomics is a sophisticated approach to measure concentrations of a large number of metabolites in biospecimens such as blood, and the metabolite profile may reflect the (patho)physiological state of individuals [3]. Previous studies using metabolomics have been able to differentiate between individuals with and without colorectal cancer [4-8].

However, few studies have investigated associations of circulating metabolites with colorectal cancer stage, and most existing studies [6, 9, 10] generally had relatively small sample sizes. Farshidfar and colleagues used an untargeted approach to identify serum metabolites related to tumour (T) and lymph node (N)-staging, among 178 patients with stage I-III colorectal cancer [6]. Thirteen out of the 40 metabolites differentiating T-stages were known biological compounds, including pyruvate and the amino acids glutamine and lysine. Four out of the 17 metabolites differentiating N-stages could be identified of which two were overlapping with metabolites distinguishing T-stages (tocopherol and nonanoic acid). Glutamine and histidine concentrations were reported to be associated with tumour stage, both metabolites tended to be lowest among patients with advanced colorectal cancer [11]. Another study showed a different serum metabolite profile, including differences in concentrations of several amino acids, between non-metastatic colorectal cancer (stage I-III, n=42) vs. metastatic colorectal cancer with liver metastases (stage IV, n=45) [9]. In addition, another small study observed that pre-treatment serum concentrations of deoxyinosine, pyridoxine, glycine, deoxycholic acid, taurocholic acid and cholesteryl esters were statistically significantly different between stage 0-I (n=8) and II-IV (n=8) colorectal cancer patients [10].

While these findings highlight the potential of metabolite profiling to identify circulating metabolites associated with colorectal cancer stage, these studies were limited by an inability to adjust for clinicodemographic and/or lifestyle factors such as age, sex, body mass index (BMI), tumour site and neo-adjuvant therapy and differences in stage classification. Given that these factors influence metabolite concentrations [12-14] and are

involved in disease progression, this could potentially account for some differences seen in studies to date. Further, prior analyses investigating metabolite profiles were often not corrected for multiple testing, increasing the chance of potential false-positive findings.

Therefore, we investigated associations of plasma concentrations of metabolites and colorectal cancer stage in a large international consortium of four cohorts among colorectal cancer patients. We compared stage II, III and IV individually with stage I colorectal cancer, as well as differences between combined stages with and without lymph node and/or distant metastases (stage III-IV vs. I-II).

Methods

Study design and populations

Data from four cohort studies within the MetaboCCC Consortium, a large consortium of European colorectal cancer cohorts established to investigate metabolic profiles across the continuum of colorectal cancer carcinogenesis, were included. The participating cohorts included: 1) the COLON study [15] from the Netherlands (ClinicalTrials.gov Identifier: NCT03191110), 2) the EnCoRe study [16] from the Netherlands (Netherlands Trial Register: 7099), 3) the Heidelberg, Germany, site of the international ColoCare Study [17] (ClinicalTrials.gov Identifier: NCT02328677) and 4) the Colorectal Cancer Study of Austria (CORSA). All cohorts were approved by local medical ethics committees and all participants provided written informed consent. In total, n=744 newly diagnosed colorectal cancer patients were included in the current study (n=197, 206, 285 and 56 from the COLON, EnCoRe, ColoCare and CORSA cohort, respectively).

The inclusion and exclusion criteria of the individual cohorts are shown in **Figure 1**. Briefly, the COLON study is an ongoing prospective cohort study among colorectal cancer patients since 2010 [15]. Participants were recruited at time of diagnosis from one academic and ten peripheral hospitals in the Netherlands. The EnCoRe study, initiated in 2012, is an ongoing prospective cohort study [16]. Colorectal cancer patients were recruited at diagnosis from one academic and two peripheral hospitals in the south-east of the Netherlands. The ColoCare Study is an ongoing, international, multi-centre prospective cohort study, which started in 2007 [17]. Colorectal cancer patients were recruited at the University Hospital of Heidelberg and the National Center for Tumor Diseases in Heidelberg, Germany. CORSA is

an ongoing study recruiting colorectal cancer patients in cooperation with the province-wide screening project Burgenland Prevention Trial of Colorectal Disease with Immunological Testing” (B-PREDICT) since 2003, using faecal occult blood testing (FOBT). FOBT-positive tested individuals subsequently received a complete colonoscopy. Additional colorectal cancer patients were recruited at diagnosis at four hospitals in Vienna.

All participants had histologically confirmed colorectal cancer and their plasma samples were assayed with a targeted metabolomics kit.

Data collection

Across all cohorts, EDTA plasma samples were collected upon patient recruitment, i.e. mostly shortly after colorectal cancer diagnosis and mostly before neo-adjuvant therapy or surgery. Plasma samples were collected and processed within four hours and stored at the corresponding study sites at -80 °C until further analysis.

Clinical data, including TNM-stage, tumour site, and treatment regimen (i.e. surgery date, whether or not patients received neo-adjuvant and adjuvant radio- and/or chemotherapy) were obtained from medical records for all cohorts. Both the pathological and clinical TNM characteristics, i.e. pTNM and cTNM, were collected from medical records. pTNM was used for staging for patients with colon cancer undergoing primary tumour resection and for rectal cancer patients without neo-adjuvant therapy, as neo-adjuvant treatment may influence pTNM staging from tumour shrinkage to enable surgery [18]. Therefore, cTNM was used to determine disease stage for all patients with neo-adjuvant therapy as well as for colon cancer patients who did not undergo surgery, as pTNM staging is not available without tumour resection. Participants were staged to overall stage (I, II, III or IV) according to the TNM classification of Malignant Tumours of the Union for International Cancer Control (8th version, 2016) [19].

Demographic and lifestyle characteristics, including age at diagnosis, sex, weight, height (to calculate BMI) and smoking status, were collected through study-specific questionnaires, except for the EnCoRe study where weight and height were measured during home visits. Data for all clinical, demographic and lifestyle characteristics were harmonized across cohorts within the MetaboCCC Consortium.

Biomarker analysis

All samples were shipped on dry ice and analysed at the International Agency for Research on Cancer (IARC) in Lyon, France, using a targeted metabolomics kit (AbsoluteIDQ™ p180 kit: BIOCRATES Life Sciences AG, Innsbruck, Austria). COLON, EnCoRe and CORSA blood samples were thawed once while ColoCare samples were not thawed before shipment to IARC. All samples of each cohort were analysed subsequently over a total of 19 batches between May and October 2016.

Figure 1 Flowchart of the total study population

The analytical method characterizes up to $n=186$ metabolites from five compound classes: $n=21$ amino acids, $n=19$ biogenic amines, $n=90$ glycerophospholipids, $n=15$ sphingolipids, $n=40$ acylcarnitines and the sum of hexose sugars. The analytical procedure has previously been described in detail [20, 21]. Briefly, liquid chromatography coupled to a tandem mass spectrometer was used, following the manufacturer's recommendations for sample preparation and analysis. Amino acids and biogenic amines were quantified by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS), whereas lipids, sugar and acylcarnitines were semi-quantified by flow injection analysis mass spectrometry (FIA-MS/MS).

Using quality control samples, metabolites with inter- or intra-batch coefficients of variation (CVs) $>20\%$ were excluded from further analysis. Subsequently, metabolites with $>20\%$ of missing values across all cohorts, including 'true' missing values as well as values below the limit of detection (LOD), or outside quantitative range of the method were excluded. This approach resulted in a total of $n=130$ metabolites for further investigation in the present study.

For these $n=130$ metabolites, imputation for values outside measurable ranges was used in case of missing values ($<20\%$) across all cohorts according to the following procedure. Values below the LOD were imputed by half of the batch-specific LOD, while values below or above the quantitative range were imputed by the lower and upper limits of quantification, respectively, following a commonly used approach for these type of data [21, 22]. An overview of all measured metabolites and the number of missing values can be found in **Supplementary Table S1**.

Statistical analysis

Clinicodemographic and lifestyle characteristics were described for the total study population, by disease stage and by cohort using descriptive analyses. Smoking status was categorized as current smoker, former smoker or never smoker. Tumour site was characterized as colon (cecum, appendix and ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon) and rectal (rectosigmoid junction and rectum) cancer.

Plasma metabolite concentrations were log transformed using the natural logarithm to normalize distributions. Thereafter, metabolite concentrations were Z-standardized to provide

a better comparison of regression coefficients across metabolites.

Multinomial logistic regression models were computed to assess the associations between concentrations of the Z-standardized metabolites separately as independent variables and for each colorectal cancer stage as dependent variable, using stage I as reference. In addition, multivariable logistic regression models were used to evaluate associations between metabolites and combined disease stages with and without lymph node and/or distant metastases (stage III-IV vs. I-II).

Analyses were adjusted for age at diagnosis (continuous), sex and BMI (continuous) as potential confounders based on published findings [13, 14]. In addition, the Principal Component Partial R-square (PC-PR2) analysis [23] was conducted to estimate the total variability in plasma metabolite concentrations attributed to potential explanatory variables including tumour site, colorectal cancer stage, smoking status, age, analytical batch, BMI, sex and cohort, results can be found in **Supplementary Figure S1**. This was done as a supporting analysis to identify potential other confounders for our analysis next to the *a priori* defined confounders, age, sex and BMI and relevant technical factors cohort and analytical batch. As can be seen in **Supplementary Figure S1**, the variables age, sex, BMI, cohort and analytical batch explained $\geq 1\%$ of the variability in metabolite levels, whereas tumour site and smoking status only explained $< 1\%$ of the variability in metabolite concentrations. We further tested whether adjustment for smoking and tumour site changed effect estimates of our main analysis. Since changes were $< 10\%$ we decided to not include smoking status and tumour site as covariates in our models.

Residuals of each metabolite concentration were calculated using linear mixed models. Metabolite concentrations were used as the independent variable, whereas random intercepts for analytical batch nested within cohorts were considered the dependent variable, as has been applied previously [24, 25]. This procedure was chosen since analytical batches were cohort-specific; using a nested variable allows to adjust for individual cohort as well as analytical batch simultaneously. In addition, this method enabled a robust adjustment for batch and cohort, by calculating residuals of plasma metabolite concentrations that are independent of analytical batch and cohort influences. Subsequently, the residuals of plasma metabolite concentrations (continuous) were included as the independent variable, and separate (II, III or IV vs. I) or combined colorectal cancer stages (III-IV vs. I-II) was defined

as the dependent variable in multinomial logistic regression models or multivariable logistic regression models, respectively.

Heterogeneity among cohorts was explored for the identified metabolites that were statistically significantly associated with individual disease stages. A random-effects meta-analysis approach [26] was used and we evaluated the I^2 index as measure for heterogeneity [27, 28]. Cohort-specific risk estimates were calculated using multinomial logistic regression models. Individual colorectal cancer stages (with stage I as reference) were added as independent variable and log transformed using the natural logarithm, Z-standardized metabolite concentrations as dependent variable. Regression models were adjusted for sex, age, body mass index (continuous) and analytical batch.

Additionally, to explore the influence of individual study cohorts, ‘leave-one-out’ multinomial logistic regression analyses were conducted comparing individual colorectal cancer stages leaving out individual study cohorts one by one.

Stratified analyses were conducted to explore sex-specific associations and differences in associations by tumour site, i.e. colon compared to rectal cancer. Multiplicative interaction by the respective variables was tested using product terms in the logistic regression models. In addition, sensitivity analyses comparing individual stages were performed: 1) excluding patients from whom blood was not collected and processed on the same day ($n=82$) and 2) excluding patients from whom blood was collected during or after any type of treatment, i.e. (neo-) adjuvant chemotherapy and/or surgery ($n=140$).

All statistical procedures were computed in R, version 3.3.6. False discovery rate (FDR) [29] was used to correct for multiple testing and findings with an FDR-corrected p -value (p_{FDR}) <0.05 were considered to be statistically significant.

Results

Study population characteristics

Characteristics for the total study population (n=744) and by disease stage at diagnosis are shown in **Table 1**. Patients had a median age of 66 years (interquartile range: 59-73), the majority of patients were men (65%) and patients presented with stage I (23%), II (28%), III (39%) or IV (10%) disease at diagnosis. Stage I patients had the highest median age and BMI compared to stage II, III and IV colorectal cancer patients. Current smoking was reported by 11-17% of stage I-IV patients. Among the study population, 58% had colon cancer, of which 28% proximal and 30% distal colon cancer, and 42% had rectal cancer. The majority of stage I colorectal cancer patients presented with distal colon cancer (42%), stage II patients had the highest percentage of proximal colon cancer (41%) while stage III and IV patients more often presented with rectal cancer, 57% and 42%, respectively. An overview of characteristics by cohort can be found in **Supplementary Table S2**, and metabolite concentrations by individual cohorts can be found in **Supplementary Table S3**.

Associations of plasma metabolite concentrations with colorectal cancer stage

Figure 2 illustrates the top 10 metabolites, ranked by p_{FDR} , associated with stage II, III or IV compared to stage I, based on multinomial logistic regression analyses. When comparing stage II with stage I colorectal cancer, none of the metabolites were significantly associated with stage after correction for multiple testing, see **Figure 2A**. Sphingomyelin C26:0 concentrations were lower among stage III compared to stage I patients (p_{FDR} : 0.038), see **Figure 2B**. Moreover, plasma concentrations of sphingomyelin C18:0 and phosphatidylcholine (diacyl) C32:0 were statistically significantly higher, whereas citrulline, histidine, phosphatidylcholine (diacyl) C34:4, phosphatidylcholine (acyl-alkyl) C40:1 and lysophosphatidylcholines (acyl) C16:0 and C17:0 were statistically significantly lower among stage IV colorectal cancer compared to stage I patients after FDR correction, see **Figure 2C**. Detailed results of all the included 130 metabolites by stage, including the mean and standard deviations of metabolite concentrations, can be found in **Supplementary Table S4**.

Heterogeneity among cohorts was evaluated for the identified metabolites that were found to be statistically significantly associated with colorectal cancer stage (as shown in **Table 2**). Overall, ORs obtained from the random-effects meta-analysis showed comparable

results with the ORs obtained from analysis within the total study population. Histidine showed high heterogeneity among cohorts ($I^2=85.3\%$, $p = 0.001$) [30]. Both COLON and CORSA showed higher histidine concentrations in stage IV colorectal cancer patients compared to stage I patients (not statistically significant), while ColoCare showed an effect size in the opposite direction for histidine (statistically significant).

‘Leave-one-out’ analyses, see **Supplementary Table S5**, showed comparable effects sizes and directions of the effect estimates compared to the analysis including the total study population. Excluding the EnCoRe study from analysis resulted in fifteen and three additional statistically significant associated glycerophospholipids and sphingolipids, respectively, with colorectal cancer stage III compared to stage I, with ORs indicating somewhat stronger associations but with a similar direction of association as in the main analysis.

When evaluating associations between metabolite concentrations and stage III-IV compared to stage I-II, fifteen out of 130 metabolites were statistically significantly associated with cancer stage prior to FDR correction, including thirteen glycerophospholipids, sphingomyelin C18:0 and citrulline. However, these associations were no longer statistically significant upon FDR correction. The ten metabolites with the lowest p_{FDR} are presented in **Table 3**. Detailed results for metabolites comparing stage III-IV vs. I-II can be found in **Supplementary Table S6**.

Results of the stratified analyses by sex and tumour site comparing individual diseases stages showed similar results compared to the analysis within the total study population (data not shown). No significant interactions by sex and tumour site were observed. Sensitivity analysis, 1) excluding patients from whom blood was not collected and processed on the same day ($n=82$) and 2) excluding patients from whom blood was collected during or after any type of treatment, i.e. (neo-) adjuvant chemotherapy and/or surgery ($n=140$), did not influence the effect sizes compared to the main analysis (data not shown). Histidine was not statistically significantly associated with stage IV compared to stage I in both sensitivity analyses.

Table 2 Baseline characteristics of the total study population and by disease stage

	Total population <i>n</i> = 744	Stage I ¹ <i>n</i> = 168	Stage II ¹ <i>n</i> = 212	Stage III ¹ <i>n</i> = 290	Stage IV ¹ <i>n</i> = 74
Male sex, n(%)	483 (64.9)	107 (63.7)	129 (60.8)	197 (67.9)	50 (67.6)
Age*, median (IQR)	66.0 (59.0-73.0)	67.0 (61.0-73.0)	68.0 (62.0-74.0)	64.0 (57.0-72.0)	60.5 (51.0-70.0)
Body mass index, kg/m ²					
Median (IQR)	26.5 (24.0-29.4)	27.1 (24.3-31.1)	26.2 (23.7-29.4)	26.5 (24.0-29.2)	25.8 (23.2-28.0)
Underweight, <18.5 n(%)	10 (1.3)	0 (0)	3 (1.4)	4 (1.4)	3 (4.1)
Normal weight, 18.5-24.9 n(%)	245 (32.9)	53 (31.5)	73 (34.4)	94 (32.4)	25 (33.8)
Overweight, 25-29.9 n(%)	330 (44.4)	72 (42.9)	89 (42.0)	134 (46.2)	35 (47.3)
Obese, ≥30 n(%)	159 (21.4)	43 (25.6)	47 (22.2)	58 (20.0)	11 (14.9)
Smoking status ² , n(%)					
Current	111 (14.9)	18 (10.7)	35 (16.5)	48 (16.6)	10 (13.5)
Former	369 (49.6)	85 (50.6)	113 (53.3)	139 (47.9)	32 (43.2)
Never	244 (32.8)	59 (35.1)	60 (28.3)	94 (32.4)	31 (41.9)
Tumour site ^{3,4,*} , n(%)					
Colon – proximal	205 (27.6)	42 (25.0)	86 (40.6)	55 (19.0)	22 (29.7)
Colon – distal	224 (30.1)	70 (41.7)	65 (30.7)	68 (23.4)	21 (28.4)
Rectal	313 (42.1)	55 (32.7)	61 (28.8)	166 (57.2)	31 (41.9)
Treatment, n(%)					
Neo-adjuvant therapy*	208 (28.0)	19 (11.3)	30 (14.2)	130 (44.8)	29 (39.2)
Surgery	732 (98.4)	167 (99.4)	210 (99.1)	283 (97.6)	72 (97.3)

¹pTNM for patients who underwent surgery, cTNM for patients with rectal cancer who received neo-adjuvant therapy and patients with colon cancer without surgery; ²Missing data for 5, 13 and 1 patients of the EnCoRe, ColoCare and CORSA study, respectively; ³Proximal consisting of: hepatic flexure, transverse colon, cecum, appendix, ascending colon; Distal consisting of: descending colon, sigmoid colon, splenic flexure; Rectal consisting of: rectosigmoid junction, rectum; ⁴Missing data for 2 patients of the CORSA study.

*Statistically significant differences between stages ($p \leq 0.05$) using Kruskal-Wallis tests or Chi-Square Tests.

Figure 2 Top 10 plasma metabolites associated with colorectal cancer stages, ranked by p -value. Black bars and symbols represent metabolites statistically significantly associated with stage after FDR correction ($p_{FDR} \leq 0.05$). Grey bars and symbols represent metabolites not statistically significantly associated with stage after FDR correction ($p_{FDR} > 0.05$). **A.** Top 10 plasma metabolites associated with stage II (n=212) colorectal cancer compared to stage I (n=168), ranked by p_{FDR} . **B.** Top 10 plasma metabolites associated with stage III (n= 290) colorectal cancer compared to stage I (n=168), ranked by p_{FDR} . **C.** Top 10 plasma metabolites associated with stage IV (n= 74) colorectal cancer compared to stage I (n=168), ranked by p_{FDR} ; Scale is logarithmic, p_{FDR} : p -value corrected for FDR.

Furthermore, sphingomyelin C26:0 was borderline significant in both sensitivity analyses when comparing stage III with stage I (both pFDR: 0.065). Lastly, both sensitivity analyses showed some additional statistically significantly associated glycerophospholipids with stage IV compared to stage I. Similar to the ‘leave-one-out’ analyses, somewhat stronger associations were observed in the sensitivity analyses but with a similar direction of association as in the main analysis.

Discussion

Associations between plasma metabolites and colorectal cancer stage were investigated in a large international consortium of four ongoing cohorts among colorectal cancer patients. We observed lower concentrations of sphingomyelin C26:0 in patients with stage III compared to stage I colorectal cancer. Plasma concentrations of sphingomyelin C18:0 and phosphatidylcholine (diacyl) C32:0 were higher in stage IV patients compared to stage I patients. Citrulline, histidine, phosphatidylcholine (diacyl) C34:4, phosphatidylcholine (acyl-alkyl) C40:1 and lysophosphatidylcholines (acyl) C16:0 and C17:0 showed lower plasma concentrations in stage IV compared to stage I patients. No statistically significant associations were observed between metabolites and combined stages with and without lymph node and/or distant metastases (stage III-IV vs. I-II).

We made the novel observation that plasma concentrations of citrulline were lower in stage IV compared to stage I colorectal cancer patients. Previously, damage in the small intestine was reported to be reflected by decreased concentrations of circulating citrulline [31]. Furthermore, citrulline plays a role in the urea cycle [32], previously linked to colorectal cancer development [25]. In addition, histidine concentrations were also lower among stage IV compared to stage I colorectal cancer patients in the current study. Histidine is linked to aspartate metabolism and is one of the amino acids entering the tricarboxylic acid cycle [33]. The tricarboxylic cycle has been reported in colorectal cancer development, as differences have been found comparing colorectal tumour tissue with normal mucosa [5, 34]. Differences in serum histidine were observed among 336 newly diagnosed colorectal cancer patients, histidine tended to be lowest among advanced colorectal cancer patients. Investigators reported systemic inflammation to be an important determinant of histidine concentrations [11]. Decreased citrulline and histidine concentrations among stage IV colorectal cancer may also result from increased hepatic uptake [35] due to possible liver metastases.

Table 2 Metabolites significantly associated with stage III or IV compared to stage I and the heterogeneity among cohorts¹

Metabolite	Total study population OR (95% CI)	COLON OR (95% CI)	EnCoRe ² OR (95% CI)	ColoCare OR (95% CI)	CORSA OR (95% CI)	Random-effects meta-analysis OR (95% CI) ³
<i>Stage III compared to stage I</i>						
Sphingomyelin C26:0	0.68 (0.55-0.84)	0.63 (0.41-0.97)	0.60 (0.37-0.97)	0.59 (0.37-0.94)	1.49 (0.63-3.53)	0.67 (0.50-0.89) (I ² = 22.1%; p = 0.28)
<i>Stage IV compared to stage I⁴</i>						
Citrulline	0.58 (0.44-0.76)	0.53 (0.20-1.40)	-	0.49 (0.31-0.78)	0.82 (0.42-1.60)	0.57 (0.40-0.81) (I ² = 0.0%; p = 0.45)
Lysophosphatidylcholine (acyl) C16:0	0.61 (0.46-0.81)	1.12 (0.30-4.15)	-	0.64 (0.44-0.92)	0.56 (0.19-1.66)	0.65 (0.47-0.91) (I ² = 0.0%; p = 0.69)
Phosphatidylcholine (diacyl) C32:0	1.62 (1.21-2.16)	1.20 (0.46-3.18)	-	1.68 (1.06-2.66)	2.09 (0.96-4.52)	1.68 (1.17-2.43) (I ² = 0.0%; p = 0.69)
Phosphatidylcholine (acyl-alkyl) C40:1	0.62 (0.47-0.82)	0.58 (0.19-1.81)	-	0.67 (0.46-0.99)	0.39 (0.11-1.35)	0.63 (0.45-0.90) (I ² = 0.0%; p = 0.71)
Sphingomyelin C18:0	1.59 (1.20-2.10)	0.66 (0.21-2.09)	-	1.88 (1.18-3.01)	2.99 (1.15-7.75)	1.70 (0.86-3.34) (I ² = 50.6%; p = 0.13)
Lysophosphatidylcholine (acyl) C17:0	0.63 (0.48-0.84)	0.88 (0.25-3.08)	-	0.70 (0.49-0.99)	0.93 (0.37-2.33)	0.73 (0.53-1.01) (I ² = 0.0%; p = 0.81)
Histidine	0.65 (0.49-0.86)	1.81 (0.42-7.70)	-	0.30 (0.16-0.55)	1.12 (0.73-1.72)	0.77 (0.27-2.22) (I ² = 85.3%; p = 0.001)
Phosphatidylcholine (diacyl) C34:4	0.66 (0.50-0.87)	0.51 (0.16-1.59)	-	0.72 (0.49-1.07)	0.68 (0.49-0.89)	0.69 (0.49-0.98) (I ² = 0.0%; p = 0.85)

Bold odds ratios (ORs) (95% CI) represent statistically significantly associated metabolites. ¹ORs per cohort were calculated using multinomial logistic regression models with colorectal cancer stage as independent variable (stage I is reference) and log transformed Z-standardized metabolite concentrations as dependent variable. Regression models were adjusted for sex, age, body mass index (continuous) and analytical batch. The use of residuals of metabolite concentrations in cohort-specific analyses was not possible since there is no correction for cohort in this analysis. Therefore, batch was added as an additional confounder in the multinomial logistic regression model; ²The EnCoRe study did not recruit stage IV patients; ³Heterogeneity among cohorts was evaluated using a random-effects meta-analysis approach and tested using the I² index with p-value; ⁴ranked by p-value of the main analysis.

Table 3 Top 10 plasma metabolites associated with colorectal cancer stage (III-IV vs. I-II), ranked by *p*-value¹

	Metabolite	Abbreviation	Stage I-II ² (<i>n</i> = 380)		Stage III-IV ² (<i>n</i> = 364)		OR ³	95% CI ⁴	<i>p</i> -value	<i>p</i> _{FDR} ⁵
			Mean	SD	Mean	SD				
1	Phosphatidylcholine (acyl-alkyl) C42:3	PC ae C42:3	0.50	± 0.14	0.47	± 0.16	0.76	(0.65-0.89)	0.0008	0.099
2	Phosphatidylcholine (acyl-alkyl) C40:1	PC ae C40:1	1.03	± 0.30	0.96	± 0.33	0.79	(0.68-0.93)	0.003	0.177
3	Phosphatidylcholine (acyl-alkyl) C42:1	PC ae C42:1	0.30	± 0.06	0.29	± 0.07	0.79	(0.68-0.93)	0.004	0.177
4	Phosphatidylcholine (acyl-alkyl) C44:6	PC ae C44:6	0.76	± 0.22	0.72	± 0.22	0.81	(0.69-0.94)	0.007	0.231
5	Phosphatidylcholine (diacyl) C42:2	PC aa C42:2	0.14	± 0.04	0.13	± 0.04	0.83	(0.71-0.97)	0.016	0.269
6	Phosphatidylcholine (acyl-alkyl) C40:3	PC ae C40:3	0.83	± 0.19	0.80	± 0.21	0.82	(0.70-0.97)	0.017	0.269
7	Sphingomyelin C18:0	SM C18:0	13.55	± 3.78	14.44	± 4.74	1.21	(1.03-1.42)	0.018	0.269
8	Phosphatidylcholine (acyl-alkyl) C38:5	PC ae C38:5	21.64	± 4.98	20.63	± 5.34	0.84	(0.72-0.97)	0.019	0.269
9	Phosphatidylcholine (acyl-alkyl) C44:3	PC ae C44:3	0.06	± 0.02	0.06	± 0.02	0.83	(0.71-0.97)	0.020	0.269
10	Phosphatidylcholine (acyl-alkyl) C34:3	PC ae C34:3	11.99	± 4.12	11.27	± 4.14	0.83	(0.72-0.97)	0.021	0.269

¹Analysed using multiple logistic regression models analysing associations of colorectal cancer stage (III-IV vs. I-II) as independent variable and the residuals obtained from linear mixed models with log transformed Z-standardized metabolite concentrations as dependent variable with random intercepts for analytical batch nested within cohort. Regression models were adjusted for sex, age, and body mass index (continuous); ²untransformed and unadjusted metabolite concentrations in $\mu\text{mol/l}$; ³Odds Ratio (*e*^b), for stage III-IV vs. I-II colorectal cancer per SD increase in transformed metabolite concentrations; ⁴Confidence Interval; ⁵*p*-value corrected for False Discovery Rate.

Next to histidine and citrulline, plasma phosphatidylcholine (diacyl) C34:4, phosphatidylcholine (acyl-alkyl) C40:1 and lysophosphatidylcholines (acyl) C16:0 and C17:0 also showed lower concentrations in stage IV patients compared to stage I colorectal cancer patients, while sphingomyelin C18:0 and phosphatidylcholine (diacyl) C32:0 showed higher concentrations. In addition, a lower plasma concentration of sphingomyelin C26:0 was found in stage III compared to stage I patients. These seven metabolites have not been associated with colorectal cancer in previous studies. Replication of our results is warranted for verification and to establish a broader understanding of metabolites and potential pathways involved in colorectal cancer progression.

Comparison of our results with previous studies investigating the relationship between metabolites and colorectal cancer stage is difficult as not many studies have been done to date, and the existing studies compared different combinations of stages (e.g. stage 0-I vs. II-IV) and used different sample matrices (serum or urine) [36]. In addition, most studies did not adjust for potential confounding factors [9-11] or multiple testing⁷ and had a small sample size [6, 9, 10]. Further, previous studies mainly used untargeted metabolomics or targeted methods covering different metabolites than in the current study.

An important strength of the current study is that we were able to investigate associations between circulating metabolites and distinct colorectal cancer stages in a large cohort of colorectal cancer patients included in the MetaboCCC consortium, with availability of harmonized data on potential confounders including clinicodemographic and lifestyle characteristics (e.g. BMI). In addition, we were able to conduct a robust correction for analytical batch and individual cohorts using the residuals of metabolite concentrations adjusted for batch nested in cohort.

It should be noted that our findings, especially for histidine, warrant careful interpretation and replication as the number of participants for some stages of disease were relatively limited (especially for stage IV with 74 patients) and not all cohorts recruited stage IV patients. When leaving out the EnCoRe study, more statistically significant associations were observed with stage III compared to stage I colorectal cancer. The results indicated somewhat stronger associations but with a similar direction of association as in the main analysis. In addition, means and standard deviations of these metabolites did not differ between the cohorts. Baseline characteristics, study design or sample collection characteristics could not explain the observed differences, since these were similar between cohorts. The EnCoRe study contributes one third

of stage III patients, and excluding this cohort, therefore, likely has a substantial influence on results. We have no further biological explanation. Still, overall heterogeneity among cohorts for the reported metabolites appeared to be minimal, except for histidine, which showed effect sizes in the opposite direction for individual cohorts. However, the findings may provide important clues for further studies focussing on mechanisms of colorectal cancer progression. Alcohol intake and fasting status have been suggested to potentially influence the concentration of certain metabolites, such as acylcarnitines and phosphatidylcholines [21, 22, 37]. We were not able to take these factors into account, because of incomplete or unavailable data in our cohorts. The issue of reverse causality should also be considered in our study because disease-related lifestyle behaviours or advanced tumour characteristics, such as lymph node involvement or disturbed liver metabolism resulting from metastases, may also be responsible for altered plasma metabolite profiles.

In conclusion, we reported associations in plasma metabolite concentrations when comparing stage III and stage IV patients individually with patients diagnosed with stage I disease, including amino acids, sphingolipids and glycerophospholipids. Our findings suggest that key metabolic pathways involving amongst other citrulline and histidine, implicated in colorectal carcinogenesis in previous studies, may be linked to disease progression as well. Further research in large sample sizes is warranted to replicate our findings, including other metabolite profiling methods, utilizing either targeted or untargeted metabolomics in different sample matrices to provide further insight into the metabolic pathways associated with colorectal cancer progression.

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Ethical approval and consent to participate The study was performed in accordance with the Declaration of Helsinki. The COLON study has been approved by the Committee on Research involving Human Subjects (region Arnhem-Nijmegen), the Netherlands; The EnCoRe study has been approved by the Medical Ethics Committee of the University Hospital Maastricht and Maastricht University, the Netherlands; The ColoCare Study has been approved by the ethics committee of the Medical Faculty at the University of Heidelberg; The CORSA Study has been approved by the institutional review board “Ethikkommission Burgenland” and by the ethical review committee of the Medical University of Vienna. All patients included in the analysis provided written informed consent.

Competing Interests Michael M. Bergmann is co-founder of Vacthera, developing oncolytic influenza viruses (www.vacthera.com), received research funding from Bristol-Myers-Squibb

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Supplementary materials

Supplementary Table S1 Metabolites measured in the BIOCRATES Absolute IDQTM p180 kit in the total study population and by individual cohort, and whether the metabolite was included for the current analysis

Total population (n= 744)										COHORTS							
Biochemical name	Abbreviation	Detec ted in ≥1 cohort	% missing	% <LO D or <LO Q*	% >ULO D **	Total % missing /outside limits	Incl. for analysis	COLON (n=197)		EnCoRe (n=206)		ColoCare (n=285)		CORSA (n=56)			
								Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits		
ACYLCARNITINES																	
Carnitine	C0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0		
Acetyl carnitine	C2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0		
Propionyl carnitine	C3	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0		
Propenoyl carnitine	C3:1	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Hydroxypropionyl carnitine	C3-OH	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Butyryl carnitine	C4	yes	0.0	0.1	0.0	0.1	yes	yes	0.0	yes	0.0	yes	0.4	yes	0.0		
Butenyl carnitine	C4:1	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Hydroxybutyryl carnitine	C4-OH (C3-DC)	yes	0.0	50.5	0.0	50.5	no	yes	45.6	yes	70.9	yes	47.7	yes	17.9		
Valeryl carnitine	C5	yes	0.0	3.2	0.0	3.2	yes	yes	3.6	yes	1.9	yes	3.5	yes	5.4		
Tiglyl carnitine	C5:1	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Glutaryl carnitine	C5-DC (C6-OH)	yes	92.5	5.5	0.0	98.0	no	no	100.0	no	100.0	no	100.0	yes	73.2		
Glutaconyl carnitine	C5-1-Dc	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Methylglutaryl carnitine	C5-M-Dc	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Hydroxyvaleryl carnitine	C5-OH (C3-DC-M)	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Hexanoyl carnitine	C6 (C4:1-DC)	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Hexenoyl carnitine	C6:1	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		
Pimelyl carnitine	C7-DC	yes	64.8	31.3	0.0	96.1	no	no	100.0	yes	92.7	no	100.0	yes	75.0		
Octanoyl carnitine	C8	yes	0.0	79.6	0.0	79.6	no	yes	85.8	yes	85.4	yes	77.5	yes	46.4		
Nonanoyl carnitine	C9	yes	0.0	86.2	0.0	86.2	no	yes	74.1	yes	85.9	yes	94.0	yes	89.3		
Decanoyl carnitine	C10	yes	0.0	50.9	0.0	50.9	no	yes	53.3	yes	52.9	yes	56.5	yes	7.1		
Decenoyl carnitine	C10:1	yes	0.0	24.5	0.0	24.5	no	yes	15.7	yes	18.9	yes	37.9	yes	7.1		
Decadienyl carnitine	C10:2	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0		

Total population (n= 744)										COHORTS					
Biochemical name	Abbreviation	Detec ted in ≥1 cohort	% missing	% <LO D or Q*	% >ULO D**	Total % missing /outside limits	Incl. for analysis	COLON (n=197)		EnCoRe (n=206)		ColoCare (n=285)		CORSA (n=56)	
								Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits
Dodecanoyl/carnitine	C12	yes	0.0	34.5	0.0	34.5	no	yes	31.5	yes	65.0	yes	19.6	yes	8.9
Dodecenoyl/carnitine	C12:1	yes	0.0	15.5	0.0	15.5	yes	yes	36.0	yes	1.9	yes	13.0	yes	5.4
Dodecanedioyl/carnitine	C12-DC	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Tetradecanoyl/carnitine	C14	yes	0.0	31.3	0.0	31.3	no	yes	22.3	yes	69.4	yes	14.4	yes	8.9
Tetradecenoyl/carnitine	C14:1	yes	0.0	0.1	0.0	0.1	yes	yes	0.0	yes	0.0	yes	0.4	yes	0.0
Hydroxytetradecenoyl- carnitine	C14:1-OH	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Tetradecadienyl/carnitine	C14:2	yes	0.0	23.1	0.0	23.1	no	yes	11.2	yes	39.8	yes	23.2	yes	3.6
Hydroxytetradecadienyl- carnitine	C14:2-OH	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Hexadecanoyl/carnitine	C16	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Hexadecenoyl/carnitine	C16:1	yes	0.0	66.3	0.0	66.3	no	yes	83.2	yes	85.0	yes	48.8	yes	26.8
Hydroxyhexadecenoyl/carnitine	C16:1-OH	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Hexadecadienyl/carnitine	C16:2	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Hydroxyhexadecadienyl- carnitine	C16:2-OH	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Hydroxyhexadecanoyl/carnitine	C16-OH	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Octadecanoyl/carnitine	C18	yes	0.0	1.1	0.0	1.1	yes	yes	0.5	yes	0.0	yes	2.5	yes	0.0
Octadecenoyl/carnitine	C18:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Hydroxyoctadecenoyl/carnitine	C18:1-OH	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Octadecadienyl/carnitine	C18:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0
AMINO ACIDS															
Alanine	Ala	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Arginine	Arg	yes	0.5	0.3	0.0	0.8	yes	yes	0.0	yes	0.0	yes	0.7	yes	7.1
Asparagine	Asn	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Aspartate	Asp	yes	0.5	16.3	0.0	16.8	yes	yes	5.1	yes	4.9	yes	31.9	yes	25.0
Citrulline	Cit	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Glutamine	Gln	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Glutamate	Glu	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1

Total population (n= 744)										COHORTS					
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								Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits
Glycine	Gly	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Histidine	His	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Isoleucine	Ile	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Leucine	Leu	yes	0.5	0.3	0.0	0.8	yes	yes	0.5	yes	0.0	yes	0.4	yes	7.1
Lysine	Lys	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Methionine	Met	yes	0.5	0.2	0.0	0.7	yes	yes	0.0	yes	0.0	yes	0.4	yes	7.1
Ornithine	Orn	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Phenylalanine	Phe	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Proline	Pro	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Serine	Ser	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Threonine	Thr	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Tryptophan	Trp	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Tyrosine	Tyr	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Valine	Val	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
BIOGENIC AMINES															
Acetylornithine	Ac-Orn	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
alpha-Aminoadipic acid	alpha-AAA	yes	0.5	62.8	0.0	63.3	no	yes	54.8	yes	59.7	yes	73.0	yes	57.1
Carnosine	Carnosine	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Creatinine	Creatinine	yes	0.5	0.0	0.0	0.5	yes	yes	0.0	yes	0.0	yes	0.0	yes	7.1
Histamine	Histamine	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Kynurenine	Kynurenine	yes	0.5	0.6	0.0	1.1	yes	yes	0.0	yes	0.0	yes	1.4	yes	7.1
Methioninesulfoxide	Met-SO	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Nitrotyrosine	Nitro-Tyr	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Phenylethylamine	PEA	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0
Putrescine	Putrescine	yes	0.5	36.6	0.0	37.1	yes	yes	29.4	yes	24.3	yes	52.3	yes	33.9
Sarcosine	Sarcosine	yes	0.5	0.2	0.0	0.7	yes	yes	0.0	yes	0.0	yes	0.4	yes	7.1
Serotonin	Serotonin	yes	0.5	44.3	0.0	44.8	no	yes	8.1	yes	7.3	yes	96.1	yes	50.0
Spermidine	Spermidine	yes	0.5	68.7	0.0	69.2	no	yes	56.3	yes	73.3	yes	77.5	yes	57.1
Spermine	Spermine	yes	0.5	79.7	0.0	80.2	no	yes	76.6	yes	62.6	yes	94.0	yes	87.5
Taurine	Taurine	yes	17.5	0.0	3.5	21.0	no	yes	10.7	yes	1.0	yes	0.0	yes	12.5
Total dimethylarginine	Total DMA	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0

Total population (n= 744)										COHORTS					
Biochemical name	Abbreviation	Detected in ≥1 cohort	% missing	% <LO D or Q*	% >ULO D **	Total % missing /outside limits	Incl. for analysis	COLON (n=197)		EnCoRe (n=206)		ColoCare (n=285)		CORSA (n=56)	
								Date cited	Total % missing /outside limits	Date cited	Total % missing /outside limits	Date cited	Total % missing /outside limits	Date cited	Total % missing /outside limits
Phosphatidylcholine (diacyl)	PC aa C24:0	no	100.0	0.0	0.0	100.0	no	100.0	no	100.0	no	100.0	no	100.0	100.0
Phosphatidylcholine (diacyl)	PC aa C26:0	no	100.0	0.0	0.0	100.0	no	100.0	no	100.0	no	100.0	no	100.0	100.0
Phosphatidylcholine (diacyl)	PC aa C28:1	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C30:0	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C30:2	no	100.0	0.0	0.0	100.0	no	100.0	no	100.0	no	100.0	no	100.0	100.0
Phosphatidylcholine (diacyl)	PC aa C32:0	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C32:1	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C32:2	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C32:3	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C34:1	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C34:2	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C34:3	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C34:4	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C36:0	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C36:1	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C36:2	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0
Phosphatidylcholine (diacyl)	PC aa C36:3	yes	0.0	0.0	0.0	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	0.0

COHORTS

Total population (n= 744)						COLON (n=197)			EnCoRe (n=206)			ColoCare (n=285)			CORSa (n=56)		
Biochemical name	Abbreviation	Detecte d in ≥1 cohort	% miss ing	% <LO D or <LO Q*	% >ULO D**	Total % miss ing /outside limits	Incl. for analysis	Dete cted	Total % miss ing /outside limits	Dete cted	Total % miss ing /outside limits	Dete cted	Total % miss ing /outside limits	Dete cted	Total % miss ing /outside limits	Dete cted	Total % miss ing /outside limits
Phosphatidylcholine (diacyl) C36:4	PC aa C36:4	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C36:5	PC aa C36:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C36:6	PC aa C36:6	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C38:0	PC aa C38:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C38:1	PC aa C38:1	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0	no	100.0
Phosphatidylcholine (diacyl) C38:3	PC aa C38:3	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C38:4	PC aa C38:4	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C38:5	PC aa C38:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C38:6	PC aa C38:6	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C40:1	PC aa C40:1	yes	0.0	76.9	0.0	76.9	no	yes	65.0	yes	72.8	yes	86.7	yes	83.9	yes	83.9
Phosphatidylcholine (diacyl) C40:2	PC aa C40:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C40:3	PC aa C40:3	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C40:4	PC aa C40:4	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C40:5	PC aa C40:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C40:6	PC aa C40:6	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C42:0	PC aa C42:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (diacyl) C42:1	PC aa C42:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0

Total population (n= 744)										COHORTS									
Biochemical name	Abbreviation	Detected in ≥1 cohort	% missing	% <LO D or <LO Q*	% >ULO D**	Total % missing /outside limits	Incl. for analysis	COLON (n=197)			EnCoRe (n=206)			ColoCare (n=285)			CORSa (n=56)		
								Detected	Total % missing /outside limits		Detected	Total % missing /outside limits		Detected	Total % missing /outside limits		Detected	Total % missing /outside limits	
Phosphatidylcholine (diacyl)																			
C42:2	PC aa C42:2	yes	0.0	0.9	0.0	0.9	yes	yes	0.0		yes	0.0		yes	2.5		yes	0.0	
Phosphatidylcholine (diacyl)																			
C42:4	PC aa C42:4	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (diacyl)																			
C42:5	PC aa C42:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (diacyl)																			
C42:6	PC aa C42:6	yes	3.0	4.3	0.0	7.3	yes	yes	0.0		yes	2.9		yes	8.1		yes	5.4	
Phosphatidylcholine (acyl- alkyl) C30:0	PC ae C30:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C30:1	PC ae C30:1	no	100.0	0.0	0.0	100.0	no	no	100.0		no	100.0		no	100.0		no	100.0	
Phosphatidylcholine (acyl- alkyl) C30:2	PC ae C30:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C32:1	PC ae C32:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C32:2	PC ae C32:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C34:0	PC ae C34:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C34:1	PC ae C34:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C34:2	PC ae C34:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C34:3	PC ae C34:3	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C36:0	PC ae C36:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C36:1	PC ae C36:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C36:2	PC ae C36:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C36:3	PC ae C36:3	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	

COHORTS

Total population (n= 744)

Biochemical name	Abbreviation	Detec ted in ≥1 cohort	% missing	% <LO D or Q*	% >ULO D**	Total % missing /outside limits	Incl. for analysis	COLON (n=197)			EnCoRe (n=206)			ColoCare (n=285)			CORSA (n=56)		
								Date cted	Total % missing /outside limits		Date cted	Total % missing /outside limits		Date cted	Total % missing /outside limits		Date cted	Total % missing /outside limits	
Phosphatidylcholine (acyl- alkyl) C36:4	PC ae C36:4	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C36:5	PC ae C36:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C38:0	PC ae C38:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C38:1	PC ae C38:1	no	100.0	0.0	0.0	100.0	no	no	100.0		no	100.0		no	100.0		no	100.0	
Phosphatidylcholine (acyl- alkyl) C38:2	PC ae C38:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C38:3	PC ae C38:3	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C38:4	PC ae C38:4	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C38:5	PC ae C38:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C38:6	PC ae C38:6	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C40:1	PC ae C40:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C40:2	PC ae C40:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C40:3	PC ae C40:3	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C40:4	PC ae C40:4	no	100.0	0.0	0.0	100.0	no	no	100.0		no	100.0		no	100.0		no	100.0	
Phosphatidylcholine (acyl- alkyl) C40:5	PC ae C40:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C40:6	PC ae C40:6	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C42:0	PC ae C42:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0		yes	0.0		yes	0.0		yes	0.0	
Phosphatidylcholine (acyl- alkyl) C42:1	PC ae C42:1	yes	0.0	0.9	0.0	0.9	yes	yes	0.0		yes	0.0		yes	2.5		yes	0.0	

Total population (n= 744)										COHORTS									
Biochemical name	Abbreviation	Detected in cohort	% missing	% <LO D or Q*	% >ULO D**	Total % missing /outside limits	Incl. for analysis	COLON (n=197)			EnCoRe (n=206)			ColoCare (n=285)			CORSa (n=56)		
								Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits	Date cted	Total % missing /outside limits
Phosphatidylcholine (acyl-alkyl) C42:2	PC ae C42:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (acyl-alkyl) C42:3	PC ae C42:3	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (acyl-alkyl) C42:4	PC ae C42:4	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (acyl-alkyl) C42:5	PC ae C42:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (acyl-alkyl) C44:3	PC ae C44:3	yes	2.3	0.5	0.0	2.8	yes	yes	0.0	yes	1.3	yes	0.7	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (acyl-alkyl) C44:4	PC ae C44:4	yes	0.0	0.1	0.0	0.1	yes	yes	0.0	yes	0.0	yes	0.4	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (acyl-alkyl) C44:5	PC ae C44:5	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Phosphatidylcholine (acyl-alkyl) C44:6	PC ae C44:6	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
SPHINGOLIPIDS																			
Hydroxy sphingomyelin C14:1	SM (OH) C14:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Hydroxy sphingomyelin C16:1	SM (OH) C16:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Hydroxy sphingomyelin C22:1	SM (OH) C22:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Hydroxy sphingomyelin C22:2	SM (OH) C22:2	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Hydroxy sphingomyelin C24:1	SM (OH) C24:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C16:0	SM C16:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C16:1	SM C16:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C18:0	SM C18:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C18:1	SM C18:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C20:2	SM C20:2	yes	0.0	0.3	0.0	0.3	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C22:3	SM C22:3	no	100.0	0.0	0.0	100.0	no	no	100.0	no	100.0	no	100.0	no	100.0	no	100.0	no	100.0
Sphingomyelin C24:0	SM C24:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C24:1	SM C24:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C26:0	SM C26:0	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0
Sphingomyelin C26:1	SM C26:1	yes	0.0	0.0	0.0	0.0	yes	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0	yes	0.0

Total population (n= 744)										COHORTS							
Biochemical name	Abbreviation	Detec ted in ≥1 cohort	% missing	% <LO D or <LO Q*	% >ULO D**	Total % missing /outside limits	Incl. for analysis	COLON (n=197)		EnCoRe (n=206)		ColoCare (n=285)		CORSA (n=56)			
								Dete cted	Total % missing /outside limits	Dete cted	Total % missing /outside limits	Dete cted	Total % missing /outside limits	Dete cted	Total % missing /outside limits		
HEXOSES																	
Hexoses	H1	yes	0.0	0.9	0.0	0.9	yes	yes	0.0	yes	0.0	yes	2.1	yes	1.8		
TOTAL NUMBER	186						130	147	148	146	149						

*LOD/LOQ: Level of detection/Level of quantification, LOD values are used for acylcarnitines, glycerophospho- and sphingolipids while LOQ values are used for amino acids, biogenic amines and sugar; **ULO D: Upper level of detection.

Supplementary Figure S1 Weighted and overall R_{partial}^2 for covariates showing the percentage of variability explained by each covariate separately and combined in metabolite concentrations, from the PC-PR2 analysis. Included covariates were stage (I/II/III/IV), smoking status (current/former/never), tumour site (distal/proximal/rectal), analytical batch (1-19), sex, cohort (COLON/EnCoRe/ColoCare/ CORSA), age and body mass index (continuous).

Supplementary Table S2 Baseline clinicodemographic and lifestyle characteristics of the total study population and by individual cohort

	Cohorts				Total population (n= 744)
	COLON (n= 197)	EnCoRe (n= 206)	ColoCare (n= 285)	CORSA (n= 56)	
Male sex, n(%)	116 (58.9)	134 (65.0)	190 (66.7)	43 (76.8)	483 (64.9)
Age*, median (IQR)	67.0 (62.0 – 72.0)	67.0 (60.0 – 73.8)	64.0 (55.0 – 72.0)	68.0 (58.5 – 75.3)	66.0 (59.0 – 73.0)
Body mass index*, kg/m ²					
Median* (IQR)	25.5 (23.2 – 28.0)	27.9 (25.6 – 30.8)	26.1 (23.7 – 28.7)	26.2 (23.9 – 29.5)	26.5 (24.0 – 29.4)
Underweight, <18.5 n(%)	3 (1.5)	1 (0.5)	6 (2.1)	0 (0.0)	10 (1.3)
Normal weight, 18.5-24.9 n(%)	85 (43.1)	44 (21.4)	98 (34.4)	18 (32.1)	245 (32.9)
Overweight, 25-29.9 n(%)	81 (41.1)	94 (45.6)	128 (44.9)	27 (48.2)	330 (44.4)
Obese, ≥30 n(%)	28 (14.2)	67 (32.5)	53 (18.6)	11 (19.6)	159 (21.4)
Smoking status ^{1,2} , n(%)					
Current	19 (9.6)	27 (13.1)	52 (18.2)	13 (23.2)	111 (14.9)
Former	119 (60.4)	110 (53.4)	121 (42.5)	19 (33.9)	369 (49.6)
Never	58 (29.4)	64 (31.1)	99 (34.7)	23 (41.1)	244 (32.8)
Stage of disease ^{2,3} , n(%)					
I	53 (26.9)	54 (26.2)	41 (14.4)	20 (35.7)	168 (22.6)
II	66 (33.5)	50 (24.3)	83 (29.1)	13 (23.2)	212 (28.5)
III	71 (36.0)	102 (49.5)	103 (36.1)	14 (25.0)	290 (39.0)
IV	7 (3.6)	0 (0.0)	58 (20.4)	9 (16.1)	74 (9.9)
Tumour site ^{3,4,5} , n(%)					
Colon – proximal	57 (28.9)	57 (27.7)	71 (24.9)	20 (35.7)	205 (27.6)
Colon – distal	71 (36.0)	70 (34.0)	65 (22.8)	18 (32.1)	224 (30.1)
Rectal	69 (35.0)	79 (38.3)	149 (52.3)	16 (28.6)	313 (42.1)
Treatment, n(%)					
Neo-adjuvant therapy*	61 (31.0)	60 (29.1)	86 (30.2)	1 (1.8)	208 (28.0)
Surgery*	197 (100.0)	196 (95.1)	285 (100.0)	54 (96.4)	732 (98.4)

¹Missing data for 5, 13 and 1 patients of the EnCoRe, ColoCare and CORSA study, respectively; ²pTNM for patients who underwent surgery; cTNM for patients with rectal cancer who received neo-adjuvant therapy and patients with colon cancer without surgery; ³Proximal consisting of: hepatic flexure, transverse colon, cecum, appendix, ascending colon; Distal consisting of: descending colon, sigmoid colon, splenic flexure; Rectal consisting of: rectosigmoid junction, rectum; ⁴Missing data for 2 patients of the CORSA study.

*Statistically significant differences between cohorts ($p<0.05$) using Kruskal-Wallis tests or Chi-Square Tests.

Supplementary Table S3 Plasma metabolite concentrations by individual cohort

Metabolite	Abbreviation	COLON Mean $\pm$ SD	EnCoRe Mean $\pm$ SD	ColoCare Mean $\pm$ SD	CORSA Mean $\pm$ SD
ACYLCARNITINES					
Carnitine	C0	35.90 $\pm$ 7.29	39.03 $\pm$ 8.83	35.75 $\pm$ 10.14	37.41 $\pm$ 8.70
Acetyl/carnitine	C2	5.94 $\pm$ 1.90	5.87 $\pm$ 1.96	7.82 $\pm$ 3.32	9.61 $\pm$ 3.56
Propionyl/carnitine	C3	0.46 $\pm$ 0.15	0.48 $\pm$ 0.14	0.37 $\pm$ 0.15	0.37 $\pm$ 0.12
Butyryl/carnitine	C4	0.24 $\pm$ 0.09	0.25 $\pm$ 0.10	0.21 $\pm$ 0.09	0.24 $\pm$ 0.33
Valeryl/carnitine	C5	0.17 $\pm$ 0.06	0.17 $\pm$ 0.06	0.20 $\pm$ 0.19	0.16 $\pm$ 0.08
Dodecenoyl/carnitine	C12:1	0.11 $\pm$ 0.05	0.16 $\pm$ 0.04	0.15 $\pm$ 0.06	0.19 $\pm$ 0.08
Tetradecenoyl/carnitine	C14:1	0.05 $\pm$ 0.02	0.05 $\pm$ 0.03	0.07 $\pm$ 0.04	0.11 $\pm$ 0.06
Hexadecanoyl/carnitine	C16	0.10 $\pm$ 0.03	0.10 $\pm$ 0.02	0.13 $\pm$ 0.04	0.16 $\pm$ 0.06
Octadecanoyl/carnitine	C18	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.06 $\pm$ 0.02	0.06 $\pm$ 0.02
Octadecenoyl/carnitine	C18:1	0.08 $\pm$ 0.03	0.09 $\pm$ 0.03	0.15 $\pm$ 0.08	0.15 $\pm$ 0.06
Octadecadienyl/carnitine	C18:2	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01	0.05 $\pm$ 0.04	0.06 $\pm$ 0.02
AMINO ACIDS					
Alanine	Ala	383.73 $\pm$ 100.56	411.50 $\pm$ 105.37	326.32 $\pm$ 95.61	333.21 $\pm$ 128.54
Arginine	Arg	81.27 $\pm$ 23.35	82.43 $\pm$ 23.41	50.65 $\pm$ 23.25	55.67 $\pm$ 22.15
Asparagine	Asn	43.44 $\pm$ 9.00	45.10 $\pm$ 12.36	41.40 $\pm$ 9.77	38.54 $\pm$ 11.86
Aspartate	Asp	11.87 $\pm$ 3.98	10.44 $\pm$ 3.85	7.45 $\pm$ 3.26	8.20 $\pm$ 3.31
Citrulline	Cit	36.51 $\pm$ 10.17	33.54 $\pm$ 11.35	28.28 $\pm$ 10.65	26.06 $\pm$ 11.19
Glutamine	Gln	695.48 $\pm$ 98.56	698.58 $\pm$ 109.20	664.43 $\pm$ 111.99	659.45 $\pm$ 212.02
Glutamate	Glu	79.86 $\pm$ 29.57	75.85 $\pm$ 30.43	61.51 $\pm$ 31.74	82.82 $\pm$ 49.48
Glycine	Gly	215.74 $\pm$ 59.66	219.88 $\pm$ 61.98	215.67 $\pm$ 61.27	195.00 $\pm$ 69.29
Histidine	His	71.01 $\pm$ 12.41	77.53 $\pm$ 15.41	66.81 $\pm$ 14.81	65.32 $\pm$ 19.77
Isoleucine	Ile	75.09 $\pm$ 23.99	82.47 $\pm$ 24.16	72.87 $\pm$ 23.02	66.69 $\pm$ 25.30
Leucine	Leu	133.34 $\pm$ 42.32	132.52 $\pm$ 39.79	125.92 $\pm$ 40.13	122.04 $\pm$ 37.45
Lysine	Lys	193.08 $\pm$ 41.77	204.04 $\pm$ 52.03	185.1 $\pm$ 49.11	182.45 $\pm$ 62.50
Methionine	Met	20.21 $\pm$ 5.78	21.53 $\pm$ 6.83	17.97 $\pm$ 5.87	18.09 $\pm$ 5.04
Ornithine	Orn	71.13 $\pm$ 24.53	75.33 $\pm$ 23.63	89.91 $\pm$ 32.62	69.69 $\pm$ 27.78
Phenylalanine	Phe	62.65 $\pm$ 13.62	68.78 $\pm$ 24.34	57.11 $\pm$ 12.34	56.55 $\pm$ 18.33
Proline	Pro	242.54 $\pm$ 79.23	244.50 $\pm$ 79.61	185.88 $\pm$ 65.45	177.2 $\pm$ 94.24
Serine	Ser	92.97 $\pm$ 20.36	91.11 $\pm$ 24.38	97.86 $\pm$ 22.19	95.41 $\pm$ 33.68
Threonine	Thr	108.73 $\pm$ 27.63	115.66 $\pm$ 31.86	106.99 $\pm$ 30.85	94.83 $\pm$ 32.85

Metabolite	Abbreviation	COLON Mean ± SD	EnCoRe Mean ± SD	ColoCare Mean ± SD	CORSA Mean ± SD
Tryptophan	Trp	53.03 ± 10.97	56.57 ± 13.10	47.45 ± 15.57	48.44 ± 16.59
Tyrosine	Tyr	74.57 ± 24.06	73.16 ± 19.21	61.50 ± 17.17	57.03 ± 19.05
Valine	Val	241.4 ± 64.04	233.04 ± 54.47	222.68 ± 57.69	212.41 ± 77.64
BIOGENIC AMINES					
Creatinine	Creatinine	74.99 ± 18.89	83.66 ± 20.72	79.90 ± 31.40	78.96 ± 26.52
Kynurenine	Kynurenine	2.68 ± 0.74	2.94 ± 0.87	2.54 ± 1.00	2.56 ± 1.00
Sarcosine	Sarcosine	4.40 ± 1.09	6.45 ± 2.93	2.33 ± 1.15	3.71 ± 1.58
Trans 4-Hydroxyproline	t4-OH-Pro	10.18 ± 4.99	11.91 ± 7.36	8.70 ± 4.47	9.04 ± 12.21
Asymmetric dimethylarginine	ADMA	0.46 ± 0.10	0.51 ± 0.13	0.47 ± 0.09	0.47 ± 0.14
Symmetric dimethylarginine	SDMA	0.46 ± 0.10	0.50 ± 0.16	0.51 ± 0.18	0.54 ± 0.27
GLYCEROPHOSPHOLIPIDS					
Lysophosphatidylcholine (acyl) C16:0	lysoPC a C16:0	90.32 ± 20.03	98.23 ± 21.92	88.26 ± 32.28	107.38 ± 31.42
Lysophosphatidylcholine (acyl) C16:1	lysoPC a C16:1	2.57 ± 0.93	2.65 ± 0.82	2.42 ± 1.06	2.68 ± 1.14
Lysophosphatidylcholine (acyl) C17:0	lysoPC a C17:0	1.56 ± 0.48	1.60 ± 0.51	1.51 ± 0.70	1.63 ± 0.59
Lysophosphatidylcholine (acyl) C18:0	lysoPC a C18:0	26.31 ± 6.84	28.42 ± 7.59	23.67 ± 10.18	28.62 ± 8.75
Lysophosphatidylcholine (acyl) C18:1	lysoPC a C18:1	17.93 ± 5.34	20.65 ± 5.83	17.81 ± 7.13	18.69 ± 7.33
Lysophosphatidylcholine (acyl) C18:2	lysoPC a C18:2	29.25 ± 10.34	32.62 ± 11.96	22.27 ± 11.31	23.07 ± 10.49
Lysophosphatidylcholine (acyl) C20:3	lysoPC a C20:3	2.04 ± 0.70	2.36 ± 0.75	1.81 ± 0.83	2.12 ± 0.84
Lysophosphatidylcholine (acyl) C20:4	lysoPC a C20:4	5.88 ± 1.71	6.34 ± 1.96	5.64 ± 2.26	7.42 ± 2.51
Lysophosphatidylcholine (acyl) C28:1	lysoPC a C28:1	0.28 ± 0.08	0.22 ± 0.07	0.21 ± 0.07	0.26 ± 0.08
Phosphatidylcholine (diacyl) C28:1	PC aa C28:1	2.99 ± 0.76	2.70 ± 0.73	2.62 ± 0.71	2.58 ± 0.81
Phosphatidylcholine (diacyl) C30:0	PC aa C30:0	4.03 ± 1.32	3.91 ± 1.46	3.71 ± 1.39	3.46 ± 1.58
Phosphatidylcholine (diacyl) C32:0	PC aa C32:0	26.66 ± 5.49	26.01 ± 5.98	23.45 ± 5.29	27.25 ± 8.95
Phosphatidylcholine (diacyl) C32:1	PC aa C32:1	31.98 ± 16.08	30.12 ± 15.42	28.66 ± 15.22	28.33 ± 18.37
Phosphatidylcholine (diacyl) C32:2	PC aa C32:2	8.34 ± 3.10	7.93 ± 3.14	5.45 ± 2.85	4.96 ± 3.15
Phosphatidylcholine (diacyl) C32:3	PC aa C32:3	0.90 ± 0.25	0.80 ± 0.26	0.66 ± 0.23	0.63 ± 0.21
Phosphatidylcholine (diacyl) C34:1	PC aa C34:1	364.98 ± 82.56	339.14 ± 79.14	345.98 ± 79.43	346.16 ± 97.47
Phosphatidylcholine (diacyl) C34:2	PC aa C34:2	739.86 ± 128.59	600.02 ± 97.89	543.17 ± 103.54	586.29 ± 144.40
Phosphatidylcholine (diacyl) C34:3	PC aa C34:3	30.14 ± 10.14	27.87 ± 10.07	20.73 ± 9.92	19.80 ± 8.65
Phosphatidylcholine (diacyl) C34:4	PC aa C34:4	3.09 ± 1.18	2.98 ± 1.17	2.25 ± 1.18	2.09 ± 1.01
Phosphatidylcholine (diacyl) C36:0	PC aa C36:0	3.24 ± 1.03	2.75 ± 0.76	2.64 ± 0.90	2.66 ± 0.97
Phosphatidylcholine (diacyl) C36:1	PC aa C36:1	62.80 ± 17.75	64.36 ± 18.49	54.10 ± 18.82	52.03 ± 19.49

Metabolite	Abbreviation	COLON Mean ± SD	EnCoRe Mean ± SD	ColoCare Mean ± SD	CORSA Mean ± SD
Phosphatidyletholine (diacyl) C36:2	PC aa C36:2	384.62 ± 80.43	344.45 ± 72.99	274.54 ± 79.12	289.54 ± 96.61
Phosphatidyletholine (diacyl) C36:3	PC aa C36:3	192.32 ± 43.96	193.01 ± 46.05	159.20 ± 46.06	163.80 ± 48.08
Phosphatidyletholine (diacyl) C36:4	PC aa C36:4	290.65 ± 72.19	275.37 ± 63.28	260.46 ± 64.48	299.70 ± 75.99
Phosphatidyletholine (diacyl) C36:5	PC aa C36:5	45.63 ± 27.7	41.16 ± 19.31	32.35 ± 19.57	27.23 ± 14.00
Phosphatidyletholine (diacyl) C36:6	PC aa C36:6	1.54 ± 0.64	1.38 ± 0.56	1.04 ± 0.59	0.82 ± 0.40
Phosphatidyletholine (diacyl) C38:0	PC aa C38:0	2.88 ± 0.89	2.68 ± 0.70	2.35 ± 0.77	2.57 ± 0.89
Phosphatidyletholine (diacyl) C38:3	PC aa C38:3	53.08 ± 13.67	58.37 ± 16.26	47.32 ± 15.93	51.06 ± 15.52
Phosphatidyletholine (diacyl) C38:4	PC aa C38:4	131.10 ± 35.15	135.83 ± 34.40	119.84 ± 33.47	142.23 ± 37.06
Phosphatidyletholine (diacyl) C38:5	PC aa C38:5	66.05 ± 19.05	66.04 ± 18.03	54.62 ± 16.51	57.51 ± 14.47
Phosphatidyletholine (diacyl) C38:6	PC aa C38:6	101.14 ± 33.50	93.43 ± 26.97	83.63 ± 27.23	86.38 ± 27.65
Phosphatidyletholine (diacyl) C40:2	PC aa C40:2	0.27 ± 0.09	0.27 ± 0.09	0.21 ± 0.07	0.22 ± 0.05
Phosphatidyletholine (diacyl) C40:3	PC aa C40:3	0.37 ± 0.14	0.43 ± 0.13	0.33 ± 0.11	0.34 ± 0.09
Phosphatidyletholine (diacyl) C40:4	PC aa C40:4	3.00 ± 0.84	3.07 ± 0.80	2.85 ± 0.99	3.36 ± 0.88
Phosphatidyletholine (diacyl) C40:5	PC aa C40:5	8.24 ± 2.43	8.44 ± 2.54	7.17 ± 2.47	7.73 ± 2.04
Phosphatidyletholine (diacyl) C40:6	PC aa C40:6	28.28 ± 10.18	27.02 ± 8.49	23.06 ± 7.59	23.63 ± 7.46
Phosphatidyletholine (diacyl) C42:0	PC aa C42:0	0.35 ± 0.11	0.33 ± 0.09	0.28 ± 0.09	0.31 ± 0.10
Phosphatidyletholine (diacyl) C42:1	PC aa C42:1	0.18 ± 0.05	0.17 ± 0.04	0.14 ± 0.04	0.16 ± 0.05
Phosphatidyletholine (diacyl) C42:2	PC aa C42:2	0.16 ± 0.04	0.15 ± 0.04	0.12 ± 0.04	0.12 ± 0.03
Phosphatidyletholine (diacyl) C42:4	PC aa C42:4	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.03	0.11 ± 0.03
Phosphatidyletholine (diacyl) C42:5	PC aa C42:5	0.25 ± 0.11	0.24 ± 0.08	0.21 ± 0.07	0.21 ± 0.05
Phosphatidyletholine (diacyl) C42:6	PC aa C42:6	0.32 ± 0.10	0.35 ± 0.09	0.31 ± 0.10	0.30 ± 0.07
Phosphatidyletholine (acyl-alkyl) C30:0	PC ae C30:0	0.34 ± 0.09	0.29 ± 0.09	0.30 ± 0.11	0.31 ± 0.11
Phosphatidyletholine (acyl-alkyl) C30:2	PC ae C30:2	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.03
Phosphatidyletholine (acyl-alkyl) C32:1	PC ae C32:1	5.27 ± 1.24	4.69 ± 1.20	4.26 ± 1.07	5.14 ± 1.73
Phosphatidyletholine (acyl-alkyl) C32:2	PC ae C32:2	1.37 ± 0.36	1.11 ± 0.31	1.04 ± 0.28	1.22 ± 0.43
Phosphatidyletholine (acyl-alkyl) C34:0	PC ae C34:0	2.46 ± 0.63	2.18 ± 0.62	2.03 ± 0.66	2.13 ± 0.80
Phosphatidyletholine (acyl-alkyl) C34:1	PC ae C34:1	18.06 ± 3.9	16.39 ± 3.70	15.87 ± 4.10	16.6 ± 4.76
Phosphatidyletholine (acyl-alkyl) C34:2	PC ae C34:2	20.31 ± 5.41	19.3 ± 5.40	16.29 ± 5.99	17.27 ± 6.09
Phosphatidyletholine (acyl-alkyl) C34:3	PC ae C34:3	13.16 ± 4.00	11.93 ± 3.73	10.43 ± 4.15	11.35 ± 4.17
Phosphatidyletholine (acyl-alkyl) C36:0	PC ae C36:0	1.17 ± 0.32	0.97 ± 0.28	1.00 ± 0.29	1.18 ± 0.49
Phosphatidyletholine (acyl-alkyl) C36:1	PC ae C36:1	11.48 ± 2.69	10.92 ± 2.67	10.40 ± 2.93	9.89 ± 2.91
Phosphatidyletholine (acyl-alkyl) C36:2	PC ae C36:2	21.75 ± 5.40	19.48 ± 5.08	17.47 ± 5.75	17.08 ± 5.95
Phosphatidyletholine (acyl-alkyl) C36:3	PC ae C36:3	11.41 ± 2.88	10.63 ± 2.86	9.39 ± 3.40	9.97 ± 2.96
Phosphatidyletholine (acyl-alkyl) C36:4	PC ae C36:4	26.27 ± 6.42	24.65 ± 5.96	22.21 ± 6.93	28.14 ± 8.71

Metabolite	Abbreviation	COLON Mean $\pm$ SD	EnCoRe Mean $\pm$ SD	ColoCare Mean $\pm$ SD	CORSA Mean $\pm$ SD
Phosphatidylcholine (acyl-alkyl) C36:5	PC ae C36:5	18.31 $\pm$ 4.95	16.22 $\pm$ 4.24	14.73 $\pm$ 4.56	18.03 $\pm$ 6.02
Phosphatidylcholine (acyl-alkyl) C38:0	PC ae C38:0	2.49 $\pm$ 0.81	2.25 $\pm$ 0.70	1.82 $\pm$ 0.76	1.64 $\pm$ 0.55
Phosphatidylcholine (acyl-alkyl) C38:2	PC ae C38:2	2.20 $\pm$ 0.55	2.39 $\pm$ 0.61	1.68 $\pm$ 0.58	1.78 $\pm$ 0.56
Phosphatidylcholine (acyl-alkyl) C38:3	PC ae C38:3	4.76 $\pm$ 1.11	4.70 $\pm$ 1.12	4.16 $\pm$ 1.25	4.27 $\pm$ 1.13
Phosphatidylcholine (acyl-alkyl) C38:4	PC ae C38:4	17.20 $\pm$ 3.62	15.94 $\pm$ 3.16	14.9 $\pm$ 3.93	18.05 $\pm$ 4.39
Phosphatidylcholine (acyl-alkyl) C38:5	PC ae C38:5	21.93 $\pm$ 4.68	22.04 $\pm$ 4.64	19.55 $\pm$ 5.27	23.26 $\pm$ 6.15
Phosphatidylcholine (acyl-alkyl) C38:6	PC ae C38:6	9.24 $\pm$ 2.65	8.42 $\pm$ 2.08	7.62 $\pm$ 2.46	8.30 $\pm$ 2.90
Phosphatidylcholine (acyl-alkyl) C40:1	PC ae C40:1	1.12 $\pm$ 0.29	1.10 $\pm$ 0.28	0.86 $\pm$ 0.31	0.90 $\pm$ 0.24
Phosphatidylcholine (acyl-alkyl) C40:2	PC ae C40:2	1.57 $\pm$ 0.41	1.54 $\pm$ 0.37	1.51 $\pm$ 0.45	1.53 $\pm$ 0.50
Phosphatidylcholine (acyl-alkyl) C40:3	PC ae C40:3	0.84 $\pm$ 0.18	0.84 $\pm$ 0.19	0.78 $\pm$ 0.21	0.84 $\pm$ 0.21
Phosphatidylcholine (acyl-alkyl) C40:4	PC ae C40:4	2.21 $\pm$ 0.45	2.17 $\pm$ 0.43	1.90 $\pm$ 0.50	2.40 $\pm$ 0.55
Phosphatidylcholine (acyl-alkyl) C40:5	PC ae C40:5	3.37 $\pm$ 0.68	3.38 $\pm$ 0.67	3.14 $\pm$ 0.79	3.48 $\pm$ 0.77
Phosphatidylcholine (acyl-alkyl) C40:6	PC ae C40:6	4.78 $\pm$ 1.24	4.46 $\pm$ 0.97	4.09 $\pm$ 1.25	4.25 $\pm$ 1.21
Phosphatidylcholine (acyl-alkyl) C42:1	PC ae C42:1	0.31 $\pm$ 0.06	0.30 $\pm$ 0.06	0.27 $\pm$ 0.07	0.31 $\pm$ 0.06
Phosphatidylcholine (acyl-alkyl) C42:2	PC ae C42:2	0.42 $\pm$ 0.11	0.40 $\pm$ 0.10	0.38 $\pm$ 0.12	0.37 $\pm$ 0.10
Phosphatidylcholine (acyl-alkyl) C42:3	PC ae C42:3	0.55 $\pm$ 0.14	0.52 $\pm$ 0.14	0.43 $\pm$ 0.14	0.45 $\pm$ 0.10
Phosphatidylcholine (acyl-alkyl) C42:4	PC ae C42:4	0.69 $\pm$ 0.16	0.67 $\pm$ 0.17	0.58 $\pm$ 0.19	0.70 $\pm$ 0.20
Phosphatidylcholine (acyl-alkyl) C42:5	PC ae C42:5	1.54 $\pm$ 0.33	1.62 $\pm$ 0.35	1.45 $\pm$ 0.39	1.63 $\pm$ 0.39
Phosphatidylcholine (acyl-alkyl) C44:3	PC ae C44:3	0.06 $\pm$ 0.02	0.06 $\pm$ 0.01	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02
Phosphatidylcholine (acyl-alkyl) C44:4	PC ae C44:4	0.23 $\pm$ 0.06	0.23 $\pm$ 0.06	0.20 $\pm$ 0.06	0.23 $\pm$ 0.06
Phosphatidylcholine (acyl-alkyl) C44:5	PC ae C44:5	0.99 $\pm$ 0.27	1.01 $\pm$ 0.27	0.97 $\pm$ 0.33	1.13 $\pm$ 0.36
Phosphatidylcholine (acyl-alkyl) C44:6	PC ae C44:6	0.78 $\pm$ 0.22	0.77 $\pm$ 0.22	0.68 $\pm$ 0.22	0.80 $\pm$ 0.25

SPHINGOLIPIDS

Hydroxysphingomyelin C14:1	SM OH C14:1	0.15 $\pm$ 0.05	0.15 $\pm$ 0.06	0.14 $\pm$ 0.07	0.18 $\pm$ 0.08
Hydroxysphingomyelin C16:1	SM OH C16:1	6.12 $\pm$ 1.41	7.59 $\pm$ 1.98	6.54 $\pm$ 1.70	6.49 $\pm$ 1.99
Hydroxysphingomyelin C22:1	SM OH C22:1	13.88 $\pm$ 3.47	16.82 $\pm$ 3.95	15.49 $\pm$ 4.19	17.13 $\pm$ 4.98
Hydroxysphingomyelin C22:2	SM OH C22:2	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.02
Hydroxysphingomyelin C24:1	SM OH C24:1	0.10 $\pm$ 0.04	0.12 $\pm$ 0.04	0.12 $\pm$ 0.04	0.12 $\pm$ 0.04
Sphingomyelin C16:0	SM C16:0	3.99 $\pm$ 1.05	4.09 $\pm$ 1.07	4.26 $\pm$ 1.21	4.02 $\pm$ 1.49
Sphingomyelin C16:1	SM C16:1	1.85 $\pm$ 0.49	1.93 $\pm$ 0.50	2.09 $\pm$ 0.61	2.05 $\pm$ 0.76
Sphingomyelin C18:0	SM C18:0	4.40 $\pm$ 1.08	4.98 $\pm$ 1.29	4.51 $\pm$ 1.30	4.53 $\pm$ 1.43
Sphingomyelin C18:1	SM C18:1	3.31 $\pm$ 0.88	3.85 $\pm$ 0.97	3.90 $\pm$ 1.13	3.67 $\pm$ 1.26
Sphingomyelin C20:2	SM C20:2	0.31 $\pm$ 0.08	0.38 $\pm$ 0.10	0.34 $\pm$ 0.10	0.34 $\pm$ 0.12

Metabolite	Abbreviation	COLON Mean ± SD	EnCoRe Mean ± SD	ColoCare Mean ± SD	CORSA Mean ± SD
Sphingomyelin C24:0	SM C24:0	67.22 ± 13.62	73.97 ± 14.39	72.56 ± 15.14	75.01 ± 19.08
Sphingomyelin C24:1	SM C24:1	9.47 ± 2.22	10.49 ± 2.46	9.77 ± 2.24	10.21 ± 2.61
Sphingomyelin C26:0	SM C26:0	12.04 ± 3.29	13.46 ± 3.37	15.41 ± 4.75	15.50 ± 4.98
Sphingomyelin C26:1	SM C26:1	5.67 ± 1.65	6.35 ± 1.77	6.80 ± 2.00	6.99 ± 2.21
HEXOSSES					
Hexoses	H1	5576.10 ± 1477.29	5513.99 ± 1231.93	4843.51 ± 1904.98	5317.45 ± 1648.64

Supplementary Table S4 Results of plasma metabolites in the total study population using multinomial analysis comparing stage II, III and IV vs. stage I colorectal cancer¹

Metabolite	Abbreviat ion	Stage I				Stage II				Stage III				Stage IV				<i>P</i> _{PRK} ³	<i>P</i> _{PRK} ³	<i>P</i> ⁺ value	<i>P</i> ⁺ value			
		Mean ± SD ²	OR ³	95% CI ⁴	<i>P</i> ⁺ value	Mean ± SD ²	OR ³	95% CI ⁴	<i>P</i> ⁺ value	Mean ± SD ²	OR ³	95% CI ⁴	<i>P</i> ⁺ value	Mean ± SD ²	OR ³	95% CI ⁴	<i>P</i> ⁺ value							
ACYLCARNITINES																								
Carnitine	C0	37.44 ± 8.54	ref.			37.18 ± 8.95	1.03	(0.83-1.27)	0.790	0.962			37.08 ± 9.33	0.97	(0.80-1.18)	0.742	0.893			33.40 ± 9.10	0.74	(0.57-0.97)	0.027	0.146
Acetylcarnitine	C2	7.06 ± 2.82	ref.			7.01 ± 3.33	0.91	(0.73-1.12)	0.373	0.905			6.68 ± 2.59	0.92	(0.75-1.12)	0.404	0.665			7.23 ± 3.20	0.84	(0.63-1.12)	0.232	0.511
Propionylcarnitine	C3	0.43 ± 0.15	ref.			0.43 ± 0.15	1.05	(0.85-1.28)	0.668	0.959			0.43 ± 0.15	1.05	(0.86-1.27)	0.641	0.843			0.36 ± 0.14	0.94	(0.71-1.25)	0.668	0.857
Butyrylcarnitine	C4	0.24 ± 0.21	ref.			0.23 ± 0.11	1.08	(0.88-1.33)	0.481	0.950			0.23 ± 0.09	1.06	(0.87-1.29)	0.571	0.807			0.19 ± 0.06	0.86	(0.64-1.16)	0.324	0.602
Valeryl carnitine	C5	0.19 ± 0.13	ref.			0.17 ± 0.08	0.85	(0.69-1.05)	0.141	0.903			0.19 ± 0.15	1.01	(0.83-1.23)	0.910	0.949			0.20 ± 0.17	1.04	(0.79-1.37)	0.766	0.881
Dodecenoylcarnitine	C12:1	0.15 ± 0.06	ref.			0.14 ± 0.07	0.96	(0.78-1.19)	0.721	0.959			0.14 ± 0.06	1.02	(0.83-1.25)	0.868	0.946			0.14 ± 0.05	1.00	(0.75-1.33)	0.982	0.996
Tetradecenoylcarnitine	C14:1	0.07 ± 0.04	ref.			0.06 ± 0.04	0.95	(0.77-1.17)	0.615	0.959			0.06 ± 0.04	1.01	(0.83-1.23)	0.900	0.949			0.07 ± 0.03	1.05	(0.79-1.39)	0.741	0.876
Hexadecenoylcarnitine	C16	0.12 ± 0.04	ref.			0.11 ± 0.04	0.84	(0.68-1.04)	0.106	0.903			0.12 ± 0.04	1.02	(0.84-1.24)	0.874	0.946			0.13 ± 0.05	1.10	(0.84-1.46)	0.480	0.717
Octadecenoylcarnitine	C18	0.05 ± 0.02	ref.			0.05 ± 0.01	0.89	(0.72-1.09)	0.262	0.905			0.05 ± 0.02	1.01	(0.83-1.23)	0.932	0.954			0.06 ± 0.02	1.07	(0.81-1.42)	0.631	0.846
Octadecenoylcarnitine	C18:1	0.11 ± 0.08	ref.			0.11 ± 0.06	0.92	(0.75-1.13)	0.424	0.911			0.11 ± 0.06	0.99	(0.81-1.20)	0.897	0.949			0.14 ± 0.07	1.06	(0.81-1.39)	0.675	0.857
Octadecadienyl carnitine	C18:2	0.05 ± 0.04	ref.			0.04 ± 0.02	0.95	(0.77-1.16)	0.615	0.959			0.04 ± 0.02	0.90	(0.74-1.09)	0.283	0.529			0.05 ± 0.03	0.89	(0.67-1.17)	0.391	0.666
AMINO ACIDS																								
Alanine	Ala	376.30 ± 116.82	ref.			359.66 ± 102.93	0.97	(0.79-1.18)	0.732	0.961			374.75 ± 109.60	1.01	(0.83-1.23)	0.907	0.949			322.74 ± 89.7	0.90	(0.68-1.20)	0.487	0.719
Arginine	Arg	70.94 ± 27.17	ref.			69.19 ± 27.96	1.09	(0.88-1.34)	0.426	0.911			68.74 ± 27.71	0.98	(0.81-1.18)	0.806	0.911			54.34 ± 25.03	0.96	(0.73-1.27)	0.788	0.891
Asparagine	Asn	43.65 ± 11.03	ref.			42.81 ± 10.87	0.89	(0.72-1.09)	0.258	0.905			42.68 ± 10.27	0.86	(0.70-1.05)	0.133	0.384			40.80 ± 10.84	0.81	(0.60-1.08)	0.148	0.404
Aspartate	Asp	100.2 ± 4.60	ref.			9.70 ± 3.72	1.07	(0.87-1.32)	0.499	0.954			9.65 ± 4.12	1.02	(0.84-1.24)	0.825	0.924			7.18 ± 2.67	0.75	(0.56-1.01)	0.061	0.240
Citrulline	Cit	33.02 ± 11.68	ref.			32.95 ± 11.19	1.00	(0.81-1.24)	0.980	0.980			32.16 ± 11.34	0.95	(0.78-1.16)	0.597	0.821			23.79 ± 7.79	0.58	(0.44-0.76)	0.000	0.014
Glutamine	Gln	69.33 ± 127.23	ref.			68.31 ± 126.91	0.92	(0.75-1.14)	0.440	0.911			68.04 ± 109.60	0.90	(0.74-1.09)	0.278	0.529			65.46 ± 113.96	0.86	(0.64-1.15)	0.316	0.602
Glutamate	Glu	72.96 ± 36.06	ref.			69.80 ± 29.75	1.04	(0.85-1.29)	0.690	0.959			72.78 ± 32.83	1.12	(0.92-1.37)	0.258	0.529			72.52 ± 40.25	1.41	(1.06-1.88)	0.019	0.131
Glycine	Gly	216.58 ± 63.85	ref.			213.14 ± 62.80	0.89	(0.72-1.10)	0.280	0.905			215.75 ± 61.91	0.91	(0.75-1.11)	0.360	0.624			216.85 ± 55.01	0.92	(0.69-1.23)	0.593	0.821

Metabolite	Abbreviat ion	Stage I			Stage II			Stage III			Stage IV				
		Mean $\pm$ SD ²	OR ³	<i>p</i> -value	Mean $\pm$ SD ²	OR ³	95% CI ⁴	Mean $\pm$ SD ²	OR ³	95% CI ⁴	Mean $\pm$ SD ²	OR ³	95% CI ⁴	<i>p</i> -value	<i>p</i> _{link} ⁵
BIOGENIC AMINES															
Creatinine	Creatinine	80.01 $\pm$ 19.10	ref.	0.917	79.66 $\pm$ 24.88	0.99	(0.80-1.22)	80.73 $\pm$ 30.37	1.03	(0.85-1.25)	73.76 $\pm$ 17.81	0.89	(0.65-1.20)	0.432	0.694
Kynurenine	Kynurenine	2.67 $\pm$ 0.89	ref.	0.285	2.71 $\pm$ 0.92	1.12	(0.91-1.39)	2.71 $\pm$ 0.94	1.13	(0.92-1.38)	2.54 $\pm$ 0.84	1.31	(0.97-1.76)	0.074	0.275
Sarcosine	Sarcosine	4.47 $\pm$ 2.45	ref.	0.692	3.97 $\pm$ 2.17	0.96	(0.78-1.18)	4.34 $\pm$ 2.71	0.97	(0.80-1.17)	2.92 $\pm$ 1.99	1.13	(0.86-1.49)	0.366	0.652
Trans 4-Hydroxyproline	t4-OH-Pro	10.04 $\pm$ 8.24	ref.	0.287	10.12 $\pm$ 6.36	1.12	(0.91-1.38)	10.18 $\pm$ 5.76	1.14	(0.94-1.39)	8.91 $\pm$ 4.69	1.19	(0.90-1.59)	0.230	0.511
Asymmetric dimethylarginine	ADMA	0.47 $\pm$ 0.12	ref.	0.910	0.48 $\pm$ 0.11	1.01	(0.82-1.25)	0.48 $\pm$ 0.12	0.97	(0.80-1.18)	0.49 $\pm$ 0.08	1.36	(1.03-1.79)	0.033	0.148
Symmetric dimethylarginine	SDMA	0.49 $\pm$ 0.13	ref.	0.635	0.51 $\pm$ 0.20	1.05	(0.85-1.29)	0.49 $\pm$ 0.17	1.00	(0.82-1.22)	0.49 $\pm$ 0.13	1.17	(0.86-1.58)	0.321	0.602
GLYCEROPHOSPHOLIPIDS															

Metabolite	Abbreviation	Stage I			Stage II			Stage III			Stage IV		
		Mean ± SD ^a	OR ^b	p-value	Mean ± SD ^a	OR ^b	p-value	Mean ± SD ^a	OR ^b	p-value	Mean ± SD ^a	OR ^b	p-value
LysopC (acyl) C16:0	lysopC a	96.79 ± 24.66	ref.	0.034	90.40 ± 28.34	0.79	0.823	94.63 ± 26.63	0.88	0.215	85.50 ± 30.48	0.61	0.001
	lysopC a	2.63 ± 0.99	ref.	0.245	2.47 ± 0.88	0.88	0.905	2.61 ± 1.03	0.93	0.480	2.27 ± 0.92	0.69	0.007
LysopC (acyl) C17:0	lysopC a	1.64 ± 0.56	ref.	0.020	1.51 ± 0.57	0.77	0.823	1.59 ± 0.61	0.85	0.117	1.43 ± 0.62	0.63	0.001
	lysopC a	27.30 ± 7.93	ref.	0.138	25.61 ± 9.02	0.85	0.903	26.32 ± 8.87	0.84	0.108	23.51 ± 9.46	0.67	0.005
LysopC (acyl) C18:1	lysopC a	19.13 ± 6.15	ref.	0.208	18.22 ± 6.25	0.87	0.905	19.17 ± 6.77	0.91	0.360	17.18 ± 6.36	0.69	0.010
	lysopC a	28.16 ± 10.80	ref.	0.412	26.73 ± 11.74	0.91	0.911	27.84 ± 12.75	0.87	0.189	22.29 ± 11.53	0.69	0.009
LysopC (acyl) C20:3	lysopC a	2.09 ± 0.76	ref.	0.367	1.97 ± 0.76	0.91	0.905	2.14 ± 0.85	0.98	0.856	1.79 ± 0.78	0.74	0.032
	lysopC a	6.42 ± 2.23	ref.	0.124	5.88 ± 2.06	0.85	0.903	6.05 ± 2.03	0.88	0.190	5.51 ± 2.23	0.68	0.007
LysopC (acyl) C28:1	lysopC a	0.24 ± 0.08	ref.	0.143	0.23 ± 0.07	0.86	0.903	0.23 ± 0.09	0.87	0.153	0.22 ± 0.07	0.83	0.007
	PC (diacyl) C28:1	2.84 ± 0.78	ref.	0.171	2.74 ± 0.70	0.87	0.903	2.70 ± 0.78	0.82	0.044	2.69 ± 0.68	0.93	0.001
PC (diacyl) C30:0	PC aa	3.72 ± 1.33	ref.	0.674	3.79 ± 1.28	1.05	0.959	3.90 ± 1.50	1.07	0.477	3.94 ± 1.62	1.15	0.001
	PC aa	25.05 ± 5.57	ref.	0.524	25.25 ± 5.80	1.07	0.959	25.11 ± 5.86	1.04	0.667	26.73 ± 8.17	1.62	0.001
PC (diacyl) C32:1	PC aa	29.56 ± 14.34	ref.	0.674	29.32 ± 14.34	1.05	0.959	31.04 ± 16.93	1.11	0.278	28.02 ± 12.42	1.02	0.873
	PC (diacyl) C32:2	7.21 ± 3.40	ref.	0.723	6.80 ± 2.97	0.96	0.959	7.08 ± 3.49	0.91	0.370	5.45 ± 3.10	0.77	0.071
PC (diacyl) C32:3	PC aa	0.80 ± 0.27	ref.	0.100	0.75 ± 0.23	0.84	0.903	0.77 ± 0.28	0.84	0.081	0.67 ± 0.23	0.81	0.001
	PC (diacyl) C34:1	344.32 ± 81.50	ref.	0.946	345.85 ± 80.62	0.99	0.970	352.57 ± 83.48	1.08	0.423	355.96 ± 82.74	1.11	0.459
PC (diacyl) C34:2	PC aa	627.25 ± 138.59	ref.	0.973	622.54 ± 140.09	1.00	0.980	611.49 ± 134.40	0.94	0.536	571.66 ± 132.54	0.93	0.599
	PC (diacyl) C34:3	25.90 ± 11.49	ref.	0.856	25.29 ± 10.03	1.02	0.962	25.68 ± 10.95	0.97	0.732	20.74 ± 9.86	0.80	0.122
PC (diacyl) C34:4	PC aa	2.85 ± 1.22	ref.	0.275	2.63 ± 1.14	0.89	0.905	2.73 ± 1.28	0.86	0.150	2.08 ± 1.12	0.66	0.003
	PC (diacyl) C36:0	2.91 ± 0.92	ref.	0.717	2.88 ± 0.95	0.96	0.959	2.77 ± 0.94	0.86	0.138	2.74 ± 0.97	0.97	0.003
PC (diacyl) C36:1	PC aa	58.67 ± 18.56	ref.	0.618	59.20 ± 18.45	1.05	0.959	60.13 ± 20.21	1.00	0.976	55.67 ± 17.73	1.00	0.995
	PC (diacyl) C36:2	330.18 ± 88.75	ref.	0.518	330.74 ± 88.25	1.07	0.959	324.20 ± 95.06	0.91	0.352	291.62 ± 87.68	0.94	0.679
PC (diacyl) C36:3	PC aa	180.07 ± 46.34	ref.	0.855	176.59 ± 44.09	0.98	0.962	181.48 ± 52.38	0.96	0.651	160.44 ± 45.68	0.81	0.142

Metabolite	Abbreviation	Stage I				Stage II				Stage III				Stage IV			
		Mean $\pm$ SD ²	OR ¹	Mean $\pm$ SD ²	OR ¹	Mean $\pm$ SD ²	OR ¹	95% CI ⁴	p-value	Mean $\pm$ SD ²	OR ¹	95% CI ⁴	p-value	Mean $\pm$ SD ²	OR ¹	95% CI ⁴	p-value
PC (diacyl) C36:4	PC aa C36:4	289.97 $\pm$ 70.02	ref.	272.80 $\pm$ 67.59	0.81	(0.66-1.00)	0.052	0.823	0.823	274.37 $\pm$ 67.55	0.83	(0.68-1.01)	0.063	255.19 $\pm$ 65.58	0.70	(0.52-0.92)	0.012
PC (diacyl) C36:5	PC aa C36:5	39.54 $\pm$ 21.18	ref.	37.98 $\pm$ 25.24	0.90	(0.73-1.11)	0.325	0.905	0.905	38.77 $\pm$ 22.25	0.94	(0.77-1.14)	0.508	30.77 $\pm$ 13.96	0.80	(0.60-1.06)	0.117
PC (diacyl) C36:6	PC aa C36:6	1.34 $\pm$ 0.65	ref.	1.23 $\pm$ 0.60	0.87	(0.70-1.07)	0.188	0.905	0.905	1.27 $\pm$ 0.65	0.86	(0.70-1.04)	0.126	0.99 $\pm$ 0.54	0.73	(0.55-0.96)	0.027
PC (diacyl) C38:0	PC aa C38:0	2.65 $\pm$ 0.75	ref.	2.65 $\pm$ 0.82	1.01	(0.82-1.25)	0.892	0.962	0.962	2.54 $\pm$ 0.85	0.86	(0.71-1.05)	0.131	2.54 $\pm$ 0.88	1.05	(0.79-1.40)	0.714
PC (diacyl) C38:3	PC aa C38:3	52.41 $\pm$ 14.27	ref.	51.87 $\pm$ 15.65	1.01	(0.82-1.25)	0.901	0.962	0.962	53.29 $\pm$ 17.41	0.97	(0.80-1.18)	0.783	48.24 $\pm$ 14.84	0.92	(0.69-1.22)	0.573
PC (diacyl) C38:4	PC aa C38:4	135.27 $\pm$ 35.05	ref.	128.16 $\pm$ 33.69	0.88	(0.71-1.08)	0.225	0.905	0.905	128.04 $\pm$ 35.87	0.83	(0.68-1.01)	0.063	120.25 $\pm$ 35.75	0.80	(0.60-1.06)	0.115
PC (diacyl) C38:5	PC aa C38:5	63.56 $\pm$ 17.12	ref.	60.23 $\pm$ 18.56	0.85	(0.69-1.04)	0.116	0.903	0.903	62.09 $\pm$ 19.13	0.89	(0.73-1.08)	0.235	53.40 $\pm$ 14.84	0.71	(0.53-0.94)	0.017
PC (diacyl) C38:6	PC aa C38:6	94.16 $\pm$ 28.79	ref.	91.33 $\pm$ 31.43	0.90	(0.74-1.11)	0.337	0.905	0.905	91.18 $\pm$ 30.46	0.91	(0.75-1.10)	0.339	84.02 $\pm$ 23.29	0.88	(0.67-1.17)	0.395
PC (diacyl) C40:2	PC aa C40:2	0.24 $\pm$ 0.08	ref.	0.25 $\pm$ 0.09	1.14	(0.92-1.41)	0.231	0.905	0.905	0.25 $\pm$ 0.09	0.96	(0.79-1.18)	0.721	0.22 $\pm$ 0.07	0.99	(0.73-1.32)	0.922
PC (diacyl) C40:3	PC aa C40:3	0.37 $\pm$ 0.13	ref.	0.37 $\pm$ 0.14	1.04	(0.84-1.29)	0.711	0.959	0.959	0.37 $\pm$ 0.13	0.96	(0.78-1.17)	0.662	0.35 $\pm$ 0.10	1.06	(0.79-1.41)	0.712
PC (diacyl) C40:4	PC aa C40:4	3.02 $\pm$ 0.81	ref.	2.96 $\pm$ 0.82	0.96	(0.78-1.18)	0.720	0.959	0.959	3.03 $\pm$ 1.02	0.96	(0.79-1.17)	0.701	2.84 $\pm$ 0.86	0.84	(0.64-1.11)	0.226
PC (diacyl) C40:5	PC aa C40:5	7.86 $\pm$ 2.26	ref.	7.67 $\pm$ 2.29	0.96	(0.78-1.18)	0.701	0.959	0.959	8.10 $\pm$ 2.83	1.05	(0.86-1.27)	0.633	7.29 $\pm$ 2.21	0.95	(0.72-1.26)	0.726
PC (diacyl) C40:6	PC aa C40:6	25.57 $\pm$ 7.73	ref.	25.96 $\pm$ 9.43	1.05	(0.86-1.29)	0.643	0.959	0.959	25.72 $\pm$ 9.46	1.00	(0.83-1.22)	0.969	24.01 $\pm$ 6.98	1.11	(0.84-1.48)	0.451
PC (diacyl) C42:0	PC aa C42:0	0.32 $\pm$ 0.10	ref.	0.32 $\pm$ 0.10	1.02	(0.83-1.27)	0.821	0.962	0.962	0.31 $\pm$ 0.10	0.85	(0.70-1.04)	0.122	0.30 $\pm$ 0.11	1.02	(0.76-1.37)	0.883
PC (diacyl) C42:1	PC aa C42:1	0.16 $\pm$ 0.05	ref.	0.16 $\pm$ 0.05	1.03	(0.83-1.28)	0.764	0.962	0.962	0.15 $\pm$ 0.05	0.83	(0.68-1.01)	0.068	0.15 $\pm$ 0.05	0.89	(0.67-1.20)	0.455
PC (diacyl) C42:2	PC aa C42:2	0.14 $\pm$ 0.04	ref.	0.14 $\pm$ 0.04	0.97	(0.78-1.20)	0.769	0.962	0.962	0.14 $\pm$ 0.04	0.83	(0.67-1.02)	0.070	0.12 $\pm$ 0.04	0.73	(0.55-0.97)	0.029
PC (diacyl) C42:4	PC aa C42:4	0.10 $\pm$ 0.02	ref.	0.10 $\pm$ 0.02	1.02	(0.83-1.27)	0.827	0.962	0.962	0.10 $\pm$ 0.03	0.82	(0.67-1.00)	0.049	0.10 $\pm$ 0.03	0.95	(0.71-1.26)	0.718
PC (diacyl) C42:5	PC aa C42:5	0.23 $\pm$ 0.07	ref.	0.23 $\pm$ 0.10	1.03	(0.84-1.27)	0.769	0.962	0.962	0.23 $\pm$ 0.09	1.03	(0.84-1.25)	0.791	0.22 $\pm$ 0.07	1.09	(0.82-1.44)	0.562
PC (diacyl) C42:6	PC aa C42:6	0.33 $\pm$ 0.09	ref.	0.31 $\pm$ 0.08	0.86	(0.70-1.07)	0.174	0.903	0.903	0.33 $\pm$ 0.11	0.92	(0.75-1.13)	0.435	0.31 $\pm$ 0.09	0.83	(0.63-1.10)	0.190
PC (acyl-alkyl) C30:0	PC ae C30:0	0.31 $\pm$ 0.10	ref.	0.31 $\pm$ 0.09	0.95	(0.77-1.17)	0.640	0.959	0.959	0.31 $\pm$ 0.11	0.95	(0.78-1.16)	0.600	0.33 $\pm$ 0.12	1.14	(0.85-1.51)	0.385
PC (acyl-alkyl) C30:2	PC ae C30:2	0.08 $\pm$ 0.02	ref.	0.07 $\pm$ 0.02	0.81	(0.66-1.00)	0.048	0.823	0.823	0.07 $\pm$ 0.02	0.79	(0.65-0.96)	0.016	0.07 $\pm$ 0.02	0.81	(0.61-1.08)	0.149
PC (acyl-alkyl) C32:1	PC ae C32:1	4.86 $\pm$ 1.36	ref.	4.73 $\pm$ 1.12	0.96	(0.78-1.19)	0.719	0.959	0.959	4.61 $\pm$ 1.29	0.85	(0.70-1.04)	0.111	4.77 $\pm$ 1.48	1.18	(0.88-1.58)	0.274
PC (acyl-alkyl) C32:2	PC ae C32:2	1.22 $\pm$ 0.38	ref.	1.16 $\pm$ 0.33	0.87	(0.71-1.08)	0.205	0.905	0.905	1.13 $\pm$ 0.34	0.81	(0.67-0.99)	0.039	1.13 $\pm$ 0.35	0.99	(0.74-1.32)	0.943

Metabolite	Abbreviat ion	Stage I			Stage II			Stage III			Stage IV							
		Mean ± SD ^a	OR ³	95% CI ⁴	p- value	p _{trisk} ⁵	Mean ± SD ^a	OR ³	95% CI ⁴	p-value	p _{trisk} ⁵	Mean ± SD ^a	OR ³	95% CI ⁴	p- value	p _{trisk} ⁵		
PC (acyl-alkyl) C34:0	PC ae C34:0	2.19 ± 0.66	ref.	2.18 ± 0.58	1.01	(0.82-1.25)	0.893	0.962	2.21 ± 0.71	1.04	(0.85-1.27)	0.704	0.872	2.20 ± 0.81	1.20	(0.90-1.60)	0.209	0.495
PC (acyl-alkyl) C34:1	PC ae C34:1	16.60 ± 4.10	ref.	16.57 ± 3.62	0.99	(0.80-1.22)	0.893	0.962	16.55 ± 4.12	0.98	(0.80-1.19)	0.834	0.927	17.40 ± 5.05	1.29	(0.96-1.73)	0.089	0.304
PC (acyl-alkyl) C34:2	PC ae C34:2	18.75 ± 5.67	ref.	18.5 ± 5.65	0.98	(0.79-1.21)	0.834	0.962	18.17 ± 6.22	0.86	(0.70-1.05)	0.140	0.386	16.85 ± 6.03	0.86	(0.64-1.14)	0.286	0.563
PC (acyl-alkyl) C34:3	PC ae C34:3	12.15 ± 4.06	ref.	11.87 ± 4.17	0.91	(0.74-1.14)	0.418	0.911	11.36 ± 4.17	0.78	(0.64-0.96)	0.019	0.192	10.89 ± 4.49	0.78	(0.58-1.04)	0.089	0.304
PC (acyl-alkyl) C36:0	PC ae C36:0	1.02 ± 0.29	ref.	1.06 ± 0.30	1.17	(0.94-1.44)	0.155	0.903	1.04 ± 0.32	1.12	(0.92-1.37)	0.253	0.529	1.14 ± 0.44	1.48	(1.11-1.97)	0.007	0.076
PC (acyl-alkyl) C36:1	PC ae C36:1	10.9 ± 2.88	ref.	10.65 ± 2.48	0.90	(0.73-1.11)	0.339	0.905	10.87 ± 3.00	0.95	(0.78-1.15)	0.586	0.819	10.63 ± 3.03	0.95	(0.72-1.27)	0.751	0.879
PC (acyl-alkyl) C36:2	PC ae C36:2	19.53 ± 5.99	ref.	19.19 ± 5.16	0.95	(0.77-1.18)	0.649	0.959	19.13 ± 6.00	0.90	(0.73-1.10)	0.285	0.529	18.07 ± 5.98	0.89	(0.67-1.20)	0.452	0.694
PC (acyl-alkyl) C36:3	PC ae C36:3	10.53 ± 3.09	ref.	10.49 ± 3.04	1.01	(0.81-1.25)	0.948	0.970	10.24 ± 3.31	0.87	(0.71-1.06)	0.169	0.430	9.61 ± 3.34	0.83	(0.62-1.10)	0.195	0.469
PC (acyl-alkyl) C36:4	PC ae C36:4	25.71 ± 7.44	ref.	24.45 ± 6.29	0.92	(0.75-1.13)	0.436	0.911	23.96 ± 7.09	0.81	(0.67-0.99)	0.040	0.250	23.08 ± 7.09	0.85	(0.64-1.12)	0.249	0.523
PC (acyl-alkyl) C36:5	PC ae C36:5	17.42 ± 5.08	ref.	16.37 ± 4.58	0.86	(0.7-1.06)	0.167	0.903	15.91 ± 4.95	0.77	(0.63-0.93)	0.009	0.143	15.46 ± 5.15	0.83	(0.62-1.10)	0.194	0.469
PC (acyl-alkyl) C38:0	PC ae C38:0	2.22 ± 0.83	ref.	2.11 ± 0.78	0.88	(0.71-1.08)	0.228	0.905	2.11 ± 0.82	0.82	(0.68-1.01)	0.057	0.297	1.79 ± 0.67	0.73	(0.54-0.97)	0.029	0.146
PC (acyl-alkyl) C38:2	PC ae C38:2	2.07 ± 0.64	ref.	2.02 ± 0.56	1.02	(0.82-1.27)	0.843	0.962	2.06 ± 0.74	0.91	(0.75-1.12)	0.379	0.639	1.77 ± 0.57	0.94	(0.70-1.25)	0.666	0.857
PC (acyl-alkyl) C38:3	PC ae C38:3	4.56 ± 1.18	ref.	4.45 ± 1.07	0.94	(0.76-1.16)	0.587	0.959	4.52 ± 1.30	0.91	(0.75-1.11)	0.358	0.624	4.20 ± 1.17	0.85	(0.64-1.12)	0.240	0.512
PC (acyl-alkyl) C38:4	PC ae C38:4	16.83 ± 3.98	ref.	15.95 ± 3.25	0.86	(0.70-1.06)	0.166	0.903	15.76 ± 3.98	0.79	(0.65-0.97)	0.023	0.202	15.50 ± 4.26	0.85	(0.64-1.13)	0.262	0.540
PC (acyl-alkyl) C38:5	PC ae C38:5	22.15 ± 5.35	ref.	21.24 ± 4.63	0.91	(0.73-1.12)	0.375	0.905	20.69 ± 5.30	0.76	(0.63-0.93)	0.008	0.143	20.43 ± 5.56	0.86	(0.65-1.14)	0.301	0.584
PC (acyl-alkyl) C40:1	PC ae C40:1	8.68 ± 2.49	ref.	8.39 ± 2.45	0.91	(0.74-1.12)	0.361	0.905	8.18 ± 2.58	0.81	(0.66-0.98)	0.034	0.244	7.89 ± 2.56	0.85	(0.64-1.13)	0.270	0.549
PC (acyl-alkyl) C40:1	PC (acyl-alkyl) C40:1	1.05 ± 0.30	ref.	1.01 ± 0.30	0.90	(0.72-1.12)	0.357	0.905	0.99 ± 0.33	0.77	(0.63-0.95)	0.014	0.189	0.83 ± 0.29	0.62	(0.47-0.82)	0.001	0.029
PC (acyl-alkyl) C40:2	PC ae C40:2	1.53 ± 0.40	ref.	1.54 ± 0.37	1.01	(0.82-1.25)	0.914	0.962	1.52 ± 0.44	0.93	(0.77-1.13)	0.482	0.737	1.62 ± 0.49	1.19	(0.89-1.59)	0.236	0.511
PC (acyl-alkyl) C40:3	PC ae C40:3	0.83 ± 0.19	ref.	0.83 ± 0.18	0.97	(0.78-1.20)	0.762	0.962	0.80 ± 0.21	0.78	(0.64-0.96)	0.018	0.190	0.81 ± 0.22	0.84	(0.62-1.12)	0.223	0.511
PC (acyl-alkyl) C40:4	PC ae C40:4	2.16 ± 0.49	ref.	2.10 ± 0.44	0.98	(0.79-1.21)	0.820	0.962	2.07 ± 0.51	0.85	(0.69-1.03)	0.099	0.369	2.03 ± 0.57	0.94	(0.70-1.24)	0.649	0.853
PC (acyl-alkyl) C40:5	PC ae C40:5	3.37 ± 0.74	ref.	3.28 ± 0.66	0.91	(0.73-1.12)	0.376	0.905	3.26 ± 0.77	0.83	(0.68-1.01)	0.069	0.305	3.31 ± 0.83	0.96	(0.72-1.28)	0.775	0.883
PC (acyl-alkyl) C40:6	PC ae C40:6	4.51 ± 1.22	ref.	4.37 ± 1.09	0.90	(0.73-1.11)	0.324	0.905	4.34 ± 1.25	0.86	(0.71-1.05)	0.146	0.394	4.30 ± 1.31	0.97	(0.73-1.30)	0.856	0.930
PC (acyl-alkyl) C42:1	PC ae C42:1	0.30 ± 0.06	ref.	0.30 ± 0.06	0.93	(0.74-1.17)	0.554	0.959	0.29 ± 0.07	0.77	(0.62-0.95)	0.015	0.189	0.27 ± 0.07	0.70	(0.53-0.92)	0.012	0.096

Metabolite	Abbreviat ion	Stage I		Stage II		Stage III		Stage IV	
		Mean $\pm$ SD ²	OR ³	Mean $\pm$ SD ²	OR ³	Mean $\pm$ SD ²	OR ³	Mean $\pm$ SD ²	OR ³
p-value									
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Metabolite	Abbreviat ion	Stage I				Stage II				Stage III				Stage IV						
		Mean ± SD ²	OR ³	Mean ± SD ²	OR ³	95% CI ⁴	p-value	p _{TRK} ⁵	Mean ± SD ²	OR ³	95% CI ⁴	p-value	p _{TRK} ⁵	Mean ± SD ²	OR ³	95% CI ⁴	p-value	p _{TRK} ⁵		
Sphingomyelin C26:1	SM C26:1	0.11 ± 0.03	ref.	0.11 ± 0.04	1.02	(0.83-1.25)	0.859	0.962			0.11 ± 0.03	0.88	(0.72-1.07)	0.190	0.449	0.13 ± 0.05	1.31	(0.99-1.73)	0.060	0.240
HEXOSSES																				
Hexoses	H1	5262.75 ± 1483.93	ref.	5284.96 ± 1658.64	1.09	(0.88-1.33)	0.432	0.911			5330.35 ± 1470.03	1.16	(0.96-1.42)	0.127	0.384	4894.55 ± 2391.15	1.03	(0.77-1.36)	0.859	0.930

Bold metabolites and corresponding p-values are significantly associated with colorectal cancer stage after FDR correction; ¹Analysed using multinomial logistic regression models analysing associations of colorectal cancer stage as independent variable (stage I is reference) and the residuals obtained from linear mixed models with log transformed Z-standardized metabolite concentrations as dependent variable with random intercepts for analytical batch nested within cohort. Regression models were adjusted for sex, age and body mass index (continuous); ²untransformed and unadjusted metabolite concentrations in µmol/l; ³Odds Ratio (eβ), for stage II, III and IV vs. I-II colorectal cancer, respectively, per SD increase in transformed metabolite concentrations; ⁴Confidence Interval; ⁵e p-value corrected for False Discovery Rate.

Supplementary Table S5 Results of the 'Leave-one-out' multinomial analyses¹ comparing stage II, III and IV vs. stage I colorectal cancer

COLON, EnCoRe and ColoCare (n=688)

Metabolite	Abbreviation	Stage I	Stage II			Stage III			Stage IV					
			OR ²	95% CI ¹	p-value	p _{FDR} ⁴	OR ²	95% CI ³	p-value	p _{FDR} ⁴	OR ²	95% CI ³	p-value	p _{FDR} ⁴
ACYLCARNITINES														
Carnitine	C0	ref.	0.96	(0.77-1.2)	0.741	0.957	0.91	(0.73-1.12)	0.359	0.648	0.68	(0.5-0.91)	0.011	0.083
Acetyl/carnitine	C2	ref.	0.88	(0.7-1.11)	0.274	0.711	0.92	(0.74-1.13)	0.427	0.694	0.87	(0.64-1.19)	0.390	0.630
Propionyl/carnitine	C3	ref.	1.02	(0.82-1.27)	0.832	0.957	0.99	(0.81-1.22)	0.945	0.971	0.92	(0.67-1.25)	0.596	0.731
Butyryl/carnitine	C4	ref.	1.11	(0.89-1.38)	0.359	0.805	1.06	(0.86-1.3)	0.584	0.810	0.87	(0.63-1.2)	0.392	0.630
Valeryl/carnitine	C5	ref.	0.83	(0.66-1.04)	0.110	0.573	1.00	(0.81-1.23)	0.993	0.993	1.08	(0.81-1.45)	0.604	0.734
Dodecenoyl/carnitine	C12:1	ref.	1.01	(0.81-1.27)	0.922	0.976	1.03	(0.84-1.28)	0.768	0.892	1.05	(0.77-1.42)	0.777	0.855
Tetradecenoyl/carnitine	C14:1	ref.	0.97	(0.77-1.21)	0.755	0.957	1.03	(0.84-1.27)	0.779	0.895	1.12	(0.82-1.52)	0.470	0.657
Hexadecanoyl/carnitine	C16	ref.	0.82	(0.65-1.02)	0.079	0.538	0.98	(0.79-1.2)	0.826	0.926	1.09	(0.81-1.47)	0.551	0.716
Octadecanoyl/carnitine	C18	ref.	0.88	(0.7-1.1)	0.255	0.711	1.00	(0.81-1.23)	0.974	0.982	1.14	(0.84-1.53)	0.405	0.639
Octadecenoyl/carnitine	C18:1	ref.	0.89	(0.72-1.11)	0.307	0.724	0.95	(0.77-1.16)	0.604	0.810	1.02	(0.76-1.36)	0.914	0.936
Octadecadienyl/carnitine	C18:2	ref.	0.93	(0.75-1.15)	0.525	0.935	0.87	(0.71-1.06)	0.169	0.428	0.83	(0.62-1.12)	0.229	0.465
AMINO ACIDS														
Alanine	Ala	ref.	0.93	(0.75-1.15)	0.510	0.928	0.95	(0.77-1.16)	0.611	0.810	0.87	(0.64-1.18)	0.367	0.618
Arginine	Arg	ref.	1.09	(0.87-1.36)	0.472	0.928	0.93	(0.75-1.14)	0.469	0.723	0.94	(0.7-1.27)	0.700	0.799
Asparagine	Asn	ref.	0.82	(0.65-1.02)	0.073	0.538	0.76	(0.61-0.94)	0.011	0.147	0.71	(0.52-0.97)	0.033	0.139
Aspartate	Asp	ref.	1.07	(0.86-1.33)	0.543	0.953	1.03	(0.83-1.26)	0.811	0.916	0.77	(0.56-1.06)	0.104	0.329

Citrulline	Cit	ref.	1.06	(0.85-1.33)	0.582	0.957	0.95	(0.77-1.17)	0.617	0.810	0.55	(0.41-0.74)	0.000	0.006
Glutamine	Gln	ref.	0.84	(0.67-1.04)	0.112	0.573	0.80	(0.65-0.99)	0.041	0.226	0.75	(0.55-1.02)	0.063	0.231
Glutamate	Glu	ref.	0.99	(0.79-1.24)	0.919	0.976	1.08	(0.88-1.34)	0.461	0.723	1.26	(0.93-1.7)	0.135	0.358
Glycine	Gly	ref.	0.88	(0.71-1.1)	0.265	0.711	0.92	(0.75-1.14)	0.449	0.721	0.87	(0.64-1.18)	0.371	0.618
Histidine	His	ref.	0.68	(0.54-0.86)	0.001	0.127	0.74	(0.59-0.92)	0.007	0.147	0.51	(0.37-0.7)	0.000	0.004
Isoleucine	Ile	ref.	1.07	(0.86-1.33)	0.566	0.957	0.96	(0.78-1.18)	0.720	0.866	0.86	(0.63-1.18)	0.347	0.598
Leucine	Leu	ref.	0.83	(0.67-1.03)	0.095	0.566	0.83	(0.67-1.02)	0.073	0.296	0.67	(0.49-0.92)	0.013	0.085
Lysine	Lys	ref.	0.83	(0.67-1.04)	0.107	0.573	0.74	(0.6-0.92)	0.006	0.147	0.65	(0.48-0.89)	0.008	0.071
Methionine	Met	ref.	0.92	(0.74-1.15)	0.489	0.928	0.98	(0.8-1.21)	0.883	0.956	0.95	(0.7-1.29)	0.760	0.844
Ornithine	Orn	ref.	1.06	(0.85-1.33)	0.577	0.957	0.93	(0.76-1.14)	0.490	0.741	0.86	(0.64-1.16)	0.332	0.583
Phenylalanine	Phe	ref.	1.08	(0.87-1.34)	0.505	0.928	1.02	(0.83-1.25)	0.849	0.936	1.02	(0.75-1.38)	0.912	0.936
Proline	Pro	ref.	1.00	(0.81-1.25)	0.976	0.983	0.95	(0.77-1.17)	0.632	0.818	0.97	(0.72-1.32)	0.860	0.908
Serine	Ser	ref.	0.92	(0.74-1.15)	0.464	0.928	0.98	(0.8-1.2)	0.841	0.934	0.90	(0.66-1.21)	0.472	0.657
Threonine	Thr	ref.	0.89	(0.71-1.1)	0.285	0.721	0.84	(0.69-1.04)	0.104	0.305	0.88	(0.65-1.18)	0.390	0.630
Tryptophan	Trp	ref.	0.85	(0.67-1.07)	0.156	0.573	0.79	(0.63-0.98)	0.032	0.219	0.64	(0.47-0.87)	0.005	0.059
Tyrosine	Tyr	ref.	0.94	(0.76-1.16)	0.567	0.957	0.90	(0.73-1.1)	0.290	0.571	0.95	(0.7-1.28)	0.723	0.811
Valine	Val	ref.	0.75	(0.59-0.94)	0.011	0.360	0.79	(0.64-0.98)	0.031	0.219	0.61	(0.44-0.84)	0.002	0.043
BIOGENIC AMINES														
Creatinine	Creatinine	ref.	0.95	(0.74-1.21)	0.679	0.957	0.94	(0.74-1.18)	0.579	0.810	0.66	(0.44-1)	0.049	0.187
Kynurenine	Kynurenine	ref.	1.02	(0.82-1.28)	0.830	0.957	1.01	(0.81-1.25)	0.950	0.971	1.09	(0.79-1.51)	0.587	0.731
Sarcosine	Sarcosine	ref.	0.93	(0.75-1.15)	0.505	0.928	0.95	(0.78-1.17)	0.644	0.818	1.10	(0.82-1.47)	0.523	0.696
Trans 4-Hydroxyproline	t4-OH-Pro	ref.	1.18	(0.94-1.47)	0.149	0.573	1.19	(0.96-1.47)	0.108	0.305	1.27	(0.93-1.72)	0.132	0.358

Asymmetric dimethyl/larginine	ADMA	ref.	0.97	(0.78-1.2)	0.756	0.957	0.89	(0.73-1.1)	0.284	0.571	1.27	(0.94-1.72)	0.126	0.351
Symmetric dimethyl/larginine	SDMA	ref.	1.06	(0.84-1.32)	0.636	0.957	0.94	(0.76-1.17)	0.590	0.810	1.10	(0.79-1.53)	0.590	0.731
GLYCEROPHOSPHOLIPIDS														
LysoPC (acyl) C16:0	lysoPC a C16:0	ref.	0.75	(0.6-0.95)	0.015	0.370	0.84	(0.68-1.05)	0.126	0.341	0.59	(0.43-0.79)	0.000	0.019
LysoPC (acyl) C16:1	lysoPC a C16:1	ref.	0.85	(0.68-1.06)	0.154	0.573	0.93	(0.75-1.15)	0.499	0.746	0.70	(0.52-0.94)	0.018	0.101
LysoPC (acyl) C17:0	lysoPC a C17:0	ref.	0.73	(0.58-0.93)	0.009	0.360	0.82	(0.65-1.02)	0.071	0.296	0.60	(0.44-0.82)	0.001	0.031
LysoPC (acyl) C18:0	lysoPC a C18:0	ref.	0.81	(0.64-1.02)	0.067	0.538	0.83	(0.66-1.03)	0.092	0.305	0.66	(0.49-0.88)	0.005	0.062
LysoPC (acyl) C18:1	lysoPC a C18:1	ref.	0.84	(0.67-1.06)	0.146	0.573	0.89	(0.72-1.11)	0.301	0.584	0.69	(0.51-0.93)	0.015	0.087
LysoPC (acyl) C18:2	lysoPC a C18:2	ref.	0.91	(0.72-1.15)	0.438	0.912	0.88	(0.7-1.09)	0.230	0.524	0.71	(0.53-0.96)	0.027	0.135
LysoPC (acyl) C20:3	lysoPC a C20:3	ref.	0.86	(0.69-1.08)	0.190	0.632	0.96	(0.77-1.19)	0.694	0.849	0.72	(0.54-0.97)	0.033	0.139
LysoPC (acyl) C20:4	lysoPC a C20:4	ref.	0.79	(0.63-0.99)	0.040	0.464	0.84	(0.68-1.03)	0.099	0.305	0.64	(0.47-0.86)	0.003	0.043
LysoPC (acyl) C28:1	lysoPC a C28:1	ref.	0.85	(0.68-1.07)	0.178	0.608	0.89	(0.72-1.1)	0.288	0.571	0.84	(0.61-1.16)	0.283	0.541
PC (diacyl) C28:1	PC aa C28:1	ref.	0.85	(0.68-1.07)	0.162	0.573	0.82	(0.67-1.02)	0.077	0.296	0.91	(0.66-1.24)	0.544	0.714
PC (diacyl) C30:0	PC aa C30:0	ref.	1.04	(0.83-1.3)	0.739	0.957	1.09	(0.89-1.35)	0.393	0.673	1.07	(0.79-1.45)	0.670	0.785
PC (diacyl) C32:0	PC aa C32:0	ref.	1.08	(0.86-1.35)	0.514	0.928	1.08	(0.88-1.34)	0.462	0.723	1.56	(1.14-2.13)	0.006	0.062
PC (diacyl) C32:1	PC aa C32:1	ref.	1.04	(0.83-1.3)	0.758	0.957	1.16	(0.94-1.44)	0.155	0.411	1.08	(0.8-1.46)	0.612	0.737
PC (diacyl) C32:2	PC aa C32:2	ref.	0.96	(0.77-1.21)	0.739	0.957	0.95	(0.77-1.18)	0.658	0.818	0.77	(0.57-1.04)	0.084	0.297
PC (diacyl) C32:3	PC aa C32:3	ref.	0.82	(0.64-1.03)	0.092	0.566	0.86	(0.69-1.07)	0.180	0.435	0.77	(0.55-1.07)	0.116	0.343
PC (diacyl) C34:1	PC aa C34:1	ref.	0.96	(0.76-1.2)	0.694	0.957	1.11	(0.9-1.37)	0.325	0.604	1.12	(0.83-1.52)	0.464	0.657
PC (diacyl) C34:2	PC aa C34:2	ref.	1.01	(0.81-1.26)	0.915	0.976	0.99	(0.8-1.22)	0.923	0.967	0.94	(0.69-1.28)	0.690	0.794
PC (diacyl) C34:3	PC aa C34:3	ref.	1.01	(0.8-1.27)	0.932	0.976	1.01	(0.81-1.25)	0.942	0.971	0.84	(0.62-1.14)	0.263	0.526
PC (diacyl) C34:4	PC aa C34:4	ref.	0.85	(0.68-1.07)	0.161	0.573	0.87	(0.7-1.08)	0.194	0.450	0.63	(0.47-0.85)	0.003	0.043

PC (diacyl) C36:0	ref.	0.97	(0.78-1.21)	0.810	0.957	0.90	(0.73-1.11)	0.331	0.606	0.96	(0.71-1.3)	0.798	0.872
PC (diacyl) C36:1	ref.	1.03	(0.82-1.29)	0.825	0.957	1.03	(0.83-1.28)	0.760	0.892	0.99	(0.72-1.35)	0.935	0.949
PC (diacyl) C36:2	ref.	1.08	(0.86-1.36)	0.496	0.928	0.97	(0.79-1.2)	0.785	0.895	0.96	(0.71-1.32)	0.819	0.887
PC (diacyl) C36:3	ref.	0.96	(0.77-1.2)	0.713	0.957	0.99	(0.8-1.22)	0.895	0.956	0.80	(0.59-1.09)	0.154	0.372
PC (diacyl) C36:4	ref.	0.76	(0.6-0.95)	0.017	0.370	0.83	(0.67-1.03)	0.083	0.301	0.66	(0.48-0.9)	0.009	0.075
PC (diacyl) C36:5	ref.	0.88	(0.71-1.1)	0.260	0.711	0.94	(0.76-1.15)	0.535	0.790	0.82	(0.6-1.11)	0.191	0.410
PC (diacyl) C36:6	ref.	0.85	(0.68-1.06)	0.145	0.573	0.87	(0.7-1.07)	0.182	0.435	0.72	(0.53-0.98)	0.036	0.142
PC (diacyl) C38:0	ref.	1.01	(0.81-1.27)	0.916	0.976	0.89	(0.72-1.1)	0.264	0.562	1.03	(0.75-1.4)	0.870	0.912
PC (diacyl) C38:3	ref.	0.96	(0.76-1.21)	0.724	0.957	0.99	(0.8-1.23)	0.956	0.971	0.88	(0.65-1.21)	0.437	0.657
PC (diacyl) C38:4	ref.	0.80	(0.63-1.01)	0.057	0.527	0.82	(0.66-1.03)	0.083	0.301	0.74	(0.54-1.02)	0.064	0.231
PC (diacyl) C38:5	ref.	0.80	(0.64-1)	0.054	0.527	0.90	(0.73-1.11)	0.323	0.604	0.71	(0.52-0.97)	0.029	0.139
PC (diacyl) C38:6	ref.	0.87	(0.69-1.08)	0.210	0.681	0.92	(0.74-1.13)	0.414	0.692	0.89	(0.66-1.22)	0.475	0.657
PC (diacyl) C40:2	ref.	1.13	(0.91-1.42)	0.269	0.711	0.95	(0.77-1.18)	0.654	0.818	0.90	(0.65-1.24)	0.518	0.696
PC (diacyl) C40:3	ref.	1.05	(0.84-1.32)	0.640	0.957	0.97	(0.78-1.2)	0.762	0.892	0.97	(0.71-1.33)	0.844	0.902
PC (diacyl) C40:4	ref.	0.91	(0.73-1.14)	0.424	0.904	0.97	(0.79-1.19)	0.762	0.892	0.77	(0.57-1.04)	0.093	0.315
PC (diacyl) C40:5	ref.	0.90	(0.72-1.13)	0.371	0.819	1.06	(0.86-1.31)	0.564	0.810	0.93	(0.69-1.26)	0.655	0.781
PC (diacyl) C40:6	ref.	0.99	(0.79-1.24)	0.939	0.976	1.01	(0.82-1.25)	0.919	0.967	1.09	(0.8-1.49)	0.579	0.731
PC (diacyl) C42:0	ref.	1.06	(0.84-1.33)	0.620	0.957	0.88	(0.71-1.09)	0.252	0.558	1.01	(0.73-1.38)	0.975	0.975
PC (diacyl) C42:1	ref.	1.03	(0.82-1.3)	0.778	0.957	0.84	(0.68-1.04)	0.107	0.305	0.85	(0.62-1.17)	0.318	0.567
PC (diacyl) C42:2	ref.	0.97	(0.77-1.23)	0.816	0.957	0.83	(0.67-1.03)	0.091	0.305	0.68	(0.5-0.92)	0.012	0.083
PC (diacyl) C42:4	ref.	0.98	(0.78-1.22)	0.829	0.957	0.79	(0.64-0.98)	0.030	0.219	0.84	(0.62-1.14)	0.273	0.529
PC (diacyl) C42:5	ref.	0.99	(0.8-1.24)	0.955	0.977	1.02	(0.83-1.25)	0.866	0.946	1.03	(0.76-1.39)	0.846	0.902
PC (diacyl) C42:6	ref.	0.84	(0.67-1.05)	0.130	0.573	0.92	(0.74-1.14)	0.424	0.694	0.80	(0.59-1.07)	0.127	0.351

PC (acyl-alkyl) C30:0	PC ae C30:0	ref.	0.96	(0.77-1.2)	0.726	0.957	0.95	(0.77-1.18)	0.656	0.818	1.09	(0.8-1.48)	0.594	0.731
PC (acyl-alkyl) C30:2	PC ae C30:2	ref.	0.77	(0.6-0.98)	0.030	0.464	0.78	(0.63-0.98)	0.035	0.219	0.78	(0.56-1.08)	0.139	0.361
PC (acyl-alkyl) C32:1	PC ae C32:1	ref.	0.99	(0.79-1.23)	0.909	0.976	0.89	(0.72-1.1)	0.272	0.569	1.16	(0.85-1.59)	0.349	0.598
PC (acyl-alkyl) C32:2	PC ae C32:2	ref.	0.88	(0.7-1.11)	0.294	0.721	0.86	(0.69-1.07)	0.164	0.426	0.99	(0.72-1.37)	0.965	0.973
PC (acyl-alkyl) C34:0	PC ae C34:0	ref.	1.00	(0.8-1.26)	0.975	0.983	1.06	(0.85-1.31)	0.615	0.810	1.14	(0.84-1.56)	0.408	0.639
PC (acyl-alkyl) C34:1	PC ae C34:1	ref.	0.98	(0.78-1.25)	0.893	0.976	1.01	(0.81-1.27)	0.897	0.956	1.32	(0.95-1.82)	0.100	0.326
PC (acyl-alkyl) C34:2	PC ae C34:2	ref.	0.97	(0.77-1.22)	0.816	0.957	0.88	(0.71-1.09)	0.257	0.558	0.85	(0.62-1.16)	0.298	0.546
PC (acyl-alkyl) C34:3	PC ae C34:3	ref.	0.95	(0.75-1.19)	0.640	0.957	0.82	(0.66-1.02)	0.077	0.296	0.77	(0.57-1.06)	0.107	0.332
PC (acyl-alkyl) C36:0	PC ae C36:0	ref.	1.19	(0.95-1.5)	0.121	0.573	1.15	(0.93-1.43)	0.184	0.435	1.42	(1.04-1.93)	0.025	0.132
PC (acyl-alkyl) C36:1	PC ae C36:1	ref.	0.85	(0.67-1.07)	0.163	0.573	0.94	(0.75-1.17)	0.569	0.810	0.90	(0.66-1.24)	0.524	0.696
PC (acyl-alkyl) C36:2	PC ae C36:2	ref.	0.94	(0.74-1.19)	0.605	0.957	0.92	(0.74-1.15)	0.473	0.723	0.88	(0.64-1.21)	0.438	0.657
PC (acyl-alkyl) C36:3	PC ae C36:3	ref.	1.00	(0.8-1.25)	0.993	0.993	0.89	(0.72-1.1)	0.284	0.571	0.82	(0.6-1.11)	0.198	0.412
PC (acyl-alkyl) C36:4	PC ae C36:4	ref.	0.88	(0.7-1.1)	0.245	0.710	0.80	(0.65-0.99)	0.042	0.226	0.81	(0.6-1.09)	0.160	0.373
PC (acyl-alkyl) C36:5	PC ae C36:5	ref.	0.84	(0.67-1.05)	0.133	0.573	0.78	(0.63-0.96)	0.019	0.192	0.78	(0.57-1.06)	0.112	0.338
PC (acyl-alkyl) C38:0	PC ae C38:0	ref.	0.84	(0.67-1.05)	0.124	0.573	0.82	(0.66-1.02)	0.075	0.296	0.71	(0.52-0.98)	0.035	0.140
PC (acyl-alkyl) C38:2	PC ae C38:2	ref.	1.03	(0.82-1.3)	0.779	0.957	0.94	(0.76-1.17)	0.599	0.810	0.91	(0.67-1.25)	0.559	0.720
PC (acyl-alkyl) C38:3	PC ae C38:3	ref.	0.89	(0.71-1.12)	0.329	0.763	0.91	(0.73-1.13)	0.373	0.655	0.80	(0.59-1.09)	0.161	0.373
PC (acyl-alkyl) C38:4	PC ae C38:4	ref.	0.80	(0.64-1.01)	0.062	0.538	0.77	(0.62-0.95)	0.016	0.192	0.80	(0.59-1.08)	0.147	0.362
PC (acyl-alkyl) C38:5	PC ae C38:5	ref.	0.87	(0.69-1.1)	0.238	0.710	0.76	(0.61-0.94)	0.011	0.147	0.81	(0.6-1.11)	0.186	0.410
PC (acyl-alkyl) C38:6	PC ae C38:6	ref.	0.88	(0.71-1.11)	0.289	0.721	0.83	(0.67-1.02)	0.077	0.296	0.81	(0.6-1.1)	0.183	0.410
PC (acyl-alkyl) C40:1	PC ae C40:1	ref.	0.88	(0.7-1.12)	0.300	0.722	0.78	(0.63-0.97)	0.029	0.219	0.62	(0.46-0.84)	0.002	0.043
PC (acyl-alkyl) C40:2	PC ae C40:2	ref.	0.98	(0.78-1.23)	0.869	0.976	0.91	(0.74-1.13)	0.415	0.692	1.07	(0.78-1.47)	0.666	0.785
PC (acyl-alkyl) C40:3	PC ae C40:3	ref.	0.96	(0.76-1.22)	0.750	0.957	0.79	(0.63-0.99)	0.038	0.225	0.79	(0.57-1.09)	0.145	0.362

PC (acyl-alkyl) C40:4	PC ae C40:4	ref.	0.95	(0.76-1.2)	0.675	0.957	0.83	(0.67-1.03)	0.096	0.305	0.88	(0.65-1.2)	0.432	0.657
PC (acyl-alkyl) C40:5	PC ae C40:5	ref.	0.91	(0.72-1.14)	0.394	0.854	0.84	(0.67-1.04)	0.104	0.305	0.94	(0.68-1.28)	0.680	0.789
PC (acyl-alkyl) C40:6	PC ae C40:6	ref.	0.87	(0.69-1.09)	0.226	0.700	0.86	(0.69-1.07)	0.171	0.428	0.94	(0.69-1.29)	0.710	0.803
PC (acyl-alkyl) C42:1	PC ae C42:1	ref.	0.91	(0.71-1.16)	0.442	0.912	0.76	(0.61-0.96)	0.019	0.192	0.67	(0.49-0.9)	0.008	0.071
PC (acyl-alkyl) C42:2	PC ae C42:2	ref.	0.98	(0.78-1.24)	0.893	0.976	0.84	(0.68-1.05)	0.121	0.335	0.84	(0.61-1.16)	0.288	0.542
PC (acyl-alkyl) C42:3	PC ae C42:3	ref.	0.97	(0.77-1.23)	0.810	0.957	0.75	(0.6-0.93)	0.009	0.147	0.69	(0.5-0.94)	0.020	0.110
PC (acyl-alkyl) C42:4	PC ae C42:4	ref.	0.97	(0.77-1.22)	0.795	0.957	0.79	(0.64-0.98)	0.034	0.219	0.89	(0.65-1.21)	0.459	0.657
PC (acyl-alkyl) C42:5	PC ae C42:5	ref.	0.96	(0.76-1.2)	0.703	0.957	0.81	(0.65-1)	0.047	0.233	0.98	(0.72-1.34)	0.898	0.934
PC (acyl-alkyl) C44:3	PC ae C44:3	ref.	1.02	(0.8-1.3)	0.856	0.976	0.83	(0.67-1.03)	0.098	0.305	0.89	(0.65-1.22)	0.470	0.657
PC (acyl-alkyl) C44:4	PC ae C44:4	ref.	0.95	(0.76-1.2)	0.680	0.957	0.81	(0.65-1)	0.048	0.233	0.85	(0.62-1.15)	0.293	0.544
PC (acyl-alkyl) C44:5	PC ae C44:5	ref.	0.98	(0.78-1.22)	0.829	0.957	0.81	(0.65-0.99)	0.044	0.231	0.89	(0.66-1.21)	0.453	0.657
PC (acyl-alkyl) C44:6	PC ae C44:6	ref.	0.95	(0.76-1.19)	0.640	0.957	0.76	(0.61-0.94)	0.010	0.147	0.80	(0.59-1.1)	0.175	0.399
SPHINGOLIPIDS														
Hydroxysphingomyelin C14:1	SM OH C14:1	ref.	1.01	(0.78-1.3)	0.955	0.977	0.90	(0.72-1.13)	0.364	0.648	1.13	(0.79-1.63)	0.497	0.681
Hydroxysphingomyelin C16:1	SM OH C16:1	ref.	0.82	(0.65-1.04)	0.096	0.566	0.77	(0.62-0.96)	0.021	0.192	0.66	(0.48-0.91)	0.012	0.083
Hydroxysphingomyelin C22:1	SM OH C22:1	ref.	1.08	(0.87-1.36)	0.479	0.928	0.94	(0.76-1.16)	0.570	0.810	1.29	(0.94-1.78)	0.120	0.346
Hydroxysphingomyelin C22:2	SM OH C22:2	ref.	0.68	(0.54-0.87)	0.002	0.127	0.63	(0.5-0.79)	0.000	0.010	0.80	(0.58-1.12)	0.192	0.410
Hydroxysphingomyelin C24:1	SM OH C24:1	ref.	0.99	(0.8-1.23)	0.933	0.976	0.84	(0.68-1.03)	0.097	0.305	1.17	(0.86-1.57)	0.318	0.567
Sphingomyelin C16:0	SM C16:0	ref.	0.86	(0.68-1.09)	0.218	0.691	0.89	(0.72-1.12)	0.322	0.604	1.24	(0.89-1.73)	0.200	0.412
Sphingomyelin C16:1	SM C16:1	ref.	0.87	(0.69-1.1)	0.246	0.710	0.95	(0.76-1.19)	0.661	0.818	1.45	(1.04-2.04)	0.030	0.139
Sphingomyelin C18:0	SM C18:0	ref.	0.81	(0.64-1.02)	0.077	0.538	0.78	(0.62-0.97)	0.026	0.219	0.67	(0.49-0.91)	0.011	0.083
Sphingomyelin C18:1	SM C18:1	ref.	0.78	(0.61-0.99)	0.043	0.464	0.74	(0.59-0.93)	0.011	0.147	0.67	(0.48-0.92)	0.014	0.087

Sphingomyelin C20:2	SM C20:2	ref.	0.78	(0.62-0.99)	0.037	0.464	0.72	(0.58-0.89)	0.003	0.147	0.71	(0.52-0.97)	0.031	0.139
Sphingomyelin C24:0	SM C24:0	ref.	1.03	(0.82-1.3)	0.784	0.957	0.91	(0.73-1.13)	0.391	0.673	1.33	(0.95-1.85)	0.095	0.315
Sphingomyelin C24:1	SM C24:1	ref.	0.77	(0.6-0.98)	0.034	0.464	0.73	(0.58-0.92)	0.007	0.147	0.88	(0.62-1.24)	0.465	0.657
Sphingomyelin C26:0	SM C26:0	ref.	0.95	(0.75-1.2)	0.669	0.957	1.04	(0.84-1.3)	0.699	0.849	1.70	(1.23-2.35)	0.001	0.031
Sphingomyelin C26:1	SM C26:1	ref.	0.76	(0.59-0.98)	0.033	0.464	0.87	(0.69-1.1)	0.258	0.558	1.22	(0.86-1.73)	0.268	0.527
HEXOSSES														
Hexoses	H1	ref.	1.11	(0.89-1.38)	0.340	0.775	1.22	(0.99-1.5)	0.061	0.282	1.25	(0.92-1.71)	0.147	0.362

COLON, EnCoRe and CORSA (n=459)

Metabolite	Abbreviation	Stage I		Stage II			Stage III			Stage IV					
				OR ²	95% CI ³	p-value	p _{FDR} ⁴	OR ²	95% CI ³	p-value	p _{FDR} ⁴	OR ²	95% CI ³	p-value	p _{FDR} ⁴
ACYLCARNITINES															
Carnitine	C0	ref.		1.04	(0.8-1.34)	0.790	0.968	1.03	(0.82-1.3)	0.804	0.983	0.83	(0.5-1.4)	0.492	0.886
Acetyl carnitine	C2	ref.		0.89	(0.69-1.15)	0.375	0.823	0.93	(0.73-1.18)	0.563	0.983	0.78	(0.45-1.35)	0.371	0.884
Propionyl carnitine	C3	ref.		1.00	(0.77-1.3)	0.997	0.997	1.20	(0.94-1.52)	0.146	0.983	1.06	(0.61-1.83)	0.831	0.960
Butyryl carnitine	C4	ref.		1.02	(0.79-1.32)	0.858	0.973	1.06	(0.84-1.34)	0.641	0.983	0.77	(0.42-1.39)	0.381	0.884
Valeryl carnitine	C5	ref.		0.91	(0.7-1.19)	0.497	0.823	1.01	(0.79-1.29)	0.955	0.983	0.99	(0.56-1.73)	0.967	0.982
Dodecenoyl carnitine	C12:1	ref.		0.92	(0.71-1.19)	0.515	0.823	1.00	(0.79-1.27)	0.991	0.991	0.92	(0.53-1.57)	0.748	0.933
Tetradecenoyl carnitine	C14:1	ref.		0.96	(0.74-1.24)	0.743	0.963	1.02	(0.8-1.28)	0.899	0.983	0.94	(0.55-1.6)	0.828	0.960
Hexadecanoyl carnitine	C16	ref.		0.90	(0.7-1.16)	0.424	0.823	1.15	(0.91-1.46)	0.231	0.983	1.13	(0.66-1.92)	0.664	0.932
Octadecanoyl carnitine	C18	ref.		0.98	(0.76-1.27)	0.903	0.973	1.15	(0.91-1.45)	0.235	0.983	0.88	(0.52-1.51)	0.656	0.932
Octadecenoyl carnitine	C18:1	ref.		1.02	(0.79-1.32)	0.854	0.973	1.10	(0.87-1.39)	0.412	0.983	1.27	(0.76-2.13)	0.366	0.884
Octadecadienyl carnitine	C18:2	ref.		1.06	(0.83-1.37)	0.641	0.922	0.97	(0.77-1.22)	0.784	0.983	1.11	(0.65-1.87)	0.705	0.933
AMINO ACIDS															
Alanine	Ala	ref.		0.91	(0.71-1.17)	0.472	0.823	1.08	(0.85-1.37)	0.532	0.983	1.07	(0.62-1.85)	0.817	0.960
Arginine	Arg	ref.		0.92	(0.71-1.19)	0.533	0.823	0.93	(0.73-1.18)	0.537	0.983	0.83	(0.49-1.39)	0.480	0.886
Asparagine	Asn	ref.		0.87	(0.67-1.13)	0.295	0.800	0.92	(0.72-1.17)	0.488	0.983	0.98	(0.56-1.71)	0.946	0.969
Aspartate	Asp	ref.		1.04	(0.81-1.33)	0.770	0.963	1.13	(0.9-1.43)	0.298	0.983	0.67	(0.39-1.14)	0.137	0.724
Citrulline	Cit	ref.		0.90	(0.7-1.16)	0.402	0.823	0.98	(0.78-1.24)	0.882	0.983	0.63	(0.38-1.05)	0.075	0.724

Glutamine	ref.	0.89	(0.69-1.16)	0.399	0.823	0.93	(0.73-1.18)	0.546	0.983	1.01	(0.58-1.75)	0.976	0.984
Glutamate	ref.	1.09	(0.84-1.4)	0.524	0.823	1.22	(0.96-1.54)	0.104	0.983	2.00	(1.12-3.57)	0.019	0.724
Glycine	ref.	0.80	(0.61-1.04)	0.094	0.552	0.92	(0.72-1.17)	0.481	0.983	1.16	(0.66-2.02)	0.606	0.918
Histidine	ref.	0.81	(0.63-1.06)	0.127	0.552	0.95	(0.74-1.21)	0.668	0.983	1.15	(0.65-2.02)	0.638	0.932
Isoleucine	ref.	1.19	(0.92-1.54)	0.196	0.671	1.03	(0.82-1.3)	0.811	0.983	1.56	(0.9-2.72)	0.115	0.724
Leucine	ref.	1.01	(0.79-1.3)	0.927	0.973	0.99	(0.78-1.25)	0.928	0.983	1.35	(0.79-2.32)	0.268	0.812
Lysine	ref.	0.96	(0.74-1.24)	0.749	0.963	0.89	(0.71-1.13)	0.345	0.983	1.21	(0.7-2.1)	0.502	0.886
Methionine	ref.	0.87	(0.68-1.13)	0.303	0.804	1.04	(0.82-1.32)	0.722	0.983	1.09	(0.64-1.88)	0.747	0.933
Ornithine	ref.	1.06	(0.82-1.37)	0.645	0.922	1.00	(0.8-1.26)	0.975	0.983	1.57	(0.91-2.69)	0.103	0.724
Phenylalanine	ref.	1.21	(0.93-1.57)	0.149	0.604	1.14	(0.91-1.44)	0.261	0.983	1.75	(1.06-2.9)	0.029	0.724
Proline	ref.	0.94	(0.73-1.22)	0.658	0.922	0.95	(0.75-1.2)	0.678	0.983	1.11	(0.64-1.91)	0.713	0.933
Serine	ref.	0.92	(0.71-1.18)	0.506	0.823	1.03	(0.81-1.31)	0.791	0.983	1.45	(0.86-2.45)	0.159	0.724
Threonine	ref.	0.86	(0.66-1.11)	0.252	0.713	0.93	(0.73-1.19)	0.571	0.983	1.16	(0.67-2.02)	0.592	0.918
Tryptophan	ref.	0.91	(0.7-1.17)	0.458	0.823	1.00	(0.78-1.27)	0.972	0.983	1.40	(0.78-2.51)	0.263	0.812
Tyrosine	ref.	0.99	(0.77-1.28)	0.942	0.973	0.93	(0.74-1.18)	0.552	0.983	1.57	(0.91-2.69)	0.106	0.724
Valine	ref.	0.97	(0.75-1.26)	0.825	0.973	0.99	(0.78-1.25)	0.908	0.983	1.58	(0.93-2.69)	0.089	0.724
BIOGENIC AMINES													
Creatinine	ref.	1.01	(0.77-1.33)	0.952	0.973	1.01	(0.78-1.31)	0.928	0.983	1.03	(0.57-1.88)	0.911	0.960
Kynurenine	ref.	1.13	(0.86-1.47)	0.379	0.823	1.31	(1.02-1.68)	0.034	0.983	1.40	(0.8-2.44)	0.243	0.802
Sarcosine	ref.	0.97	(0.75-1.25)	0.824	0.973	0.98	(0.78-1.24)	0.882	0.983	1.17	(0.69-1.98)	0.564	0.918
Trans 4-Hydroxyproline	ref.	1.11	(0.86-1.43)	0.442	0.823	0.99	(0.78-1.25)	0.921	0.983	1.15	(0.68-1.92)	0.604	0.918
Asymmetric dimethylarginine	ref.	1.01	(0.78-1.3)	0.958	0.973	1.11	(0.88-1.4)	0.366	0.983	1.35	(0.81-2.25)	0.248	0.802

Symmetric dimethylarginine	SDMA	ref.	1.07	(0.83-1.39)	0.584	0.862	1.06	(0.84-1.35)	0.609	0.983	1.35	(0.78-2.33)	0.283	0.818
GLYCEROPHOSPHOLIPIDS														
LysoPC (acyl) C16:0	lysoPC a C16:0	ref.	0.72	(0.56-0.94)	0.015	0.269	0.96	(0.75-1.21)	0.708	0.983	0.69	(0.4-1.19)	0.178	0.724
LysoPC (acyl) C16:1	lysoPC a C16:1	ref.	0.82	(0.63-1.06)	0.122	0.552	1.01	(0.8-1.27)	0.961	0.983	0.64	(0.37-1.11)	0.112	0.724
LysoPC (acyl) C17:0	lysoPC a C17:0	ref.	0.65	(0.5-0.85)	0.001	0.115	0.94	(0.74-1.19)	0.595	0.983	0.72	(0.41-1.25)	0.240	0.802
LysoPC (acyl) C18:0	lysoPC a C18:0	ref.	0.79	(0.61-1.02)	0.072	0.503	0.88	(0.69-1.12)	0.305	0.983	0.69	(0.4-1.19)	0.178	0.724
LysoPC (acyl) C18:1	lysoPC a C18:1	ref.	0.80	(0.61-1.04)	0.089	0.551	0.92	(0.72-1.17)	0.511	0.983	0.63	(0.35-1.11)	0.107	0.724
LysoPC (acyl) C18:2	lysoPC a C18:2	ref.	0.85	(0.65-1.11)	0.246	0.711	0.87	(0.68-1.11)	0.253	0.983	0.60	(0.34-1.05)	0.073	0.724
LysoPC (acyl) C20:3	lysoPC a C20:3	ref.	0.86	(0.67-1.12)	0.262	0.725	1.02	(0.81-1.3)	0.843	0.983	0.76	(0.44-1.3)	0.317	0.859
LysoPC (acyl) C20:4	lysoPC a C20:4	ref.	0.86	(0.67-1.11)	0.223	0.689	0.91	(0.72-1.15)	0.433	0.983	0.72	(0.43-1.21)	0.213	0.768
LysoPC (acyl) C28:1	lysoPC a C28:1	ref.	0.70	(0.53-0.92)	0.011	0.245	0.99	(0.77-1.27)	0.917	0.983	0.86	(0.48-1.54)	0.606	0.918
PC (diacyl) C28:1	PC aa C28:1	ref.	0.76	(0.58-0.98)	0.037	0.381	0.90	(0.71-1.14)	0.386	0.983	1.05	(0.6-1.84)	0.856	0.960
PC (diacyl) C30:0	PC aa C30:0	ref.	0.98	(0.76-1.27)	0.889	0.973	1.13	(0.89-1.43)	0.306	0.983	1.46	(0.85-2.5)	0.173	0.724
PC (diacyl) C32:0	PC aa C32:0	ref.	1.10	(0.85-1.43)	0.470	0.823	1.11	(0.87-1.4)	0.400	0.983	1.85	(1.09-3.13)	0.022	0.724
PC (diacyl) C32:1	PC aa C32:1	ref.	1.02	(0.79-1.32)	0.881	0.973	1.13	(0.89-1.42)	0.316	0.983	0.97	(0.57-1.65)	0.904	0.960
PC (diacyl) C32:2	PC aa C32:2	ref.	0.81	(0.63-1.06)	0.124	0.552	0.89	(0.7-1.13)	0.347	0.983	0.79	(0.46-1.37)	0.411	0.884
PC (diacyl) C32:3	PC aa C32:3	ref.	0.68	(0.51-0.9)	0.007	0.212	0.84	(0.66-1.08)	0.186	0.983	0.79	(0.44-1.44)	0.443	0.886
PC (diacyl) C34:1	PC aa C34:1	ref.	0.96	(0.74-1.24)	0.747	0.963	1.10	(0.87-1.39)	0.431	0.983	1.10	(0.64-1.89)	0.736	0.933
PC (diacyl) C34:2	PC aa C34:2	ref.	0.96	(0.75-1.24)	0.764	0.963	0.99	(0.78-1.25)	0.917	0.983	1.03	(0.59-1.78)	0.929	0.960
PC (diacyl) C34:3	PC aa C34:3	ref.	0.94	(0.72-1.22)	0.642	0.922	0.95	(0.75-1.21)	0.666	0.983	0.75	(0.43-1.29)	0.294	0.832
PC (diacyl) C34:4	PC aa C34:4	ref.	0.79	(0.6-1.02)	0.074	0.503	0.84	(0.66-1.08)	0.171	0.983	0.67	(0.4-1.13)	0.135	0.724
PC (diacyl) C36:0	PC aa C36:0	ref.	0.95	(0.74-1.23)	0.695	0.941	0.94	(0.74-1.19)	0.596	0.983	1.05	(0.62-1.79)	0.861	0.960

PC (diacyl) C36:1	ref.	1.01	(0.77-1.32)	0.929	0.973	1.01	(0.79-1.29)	0.937	0.983	0.97	(0.55-1.7)	0.907	0.960
PC (diacyl) C36:2	ref.	1.02	(0.79-1.33)	0.863	0.973	0.92	(0.73-1.18)	0.519	0.983	1.00	(0.57-1.74)	0.989	0.989
PC (diacyl) C36:3	ref.	0.90	(0.69-1.17)	0.422	0.823	1.02	(0.8-1.3)	0.882	0.983	0.83	(0.48-1.45)	0.517	0.888
PC (diacyl) C36:4	ref.	0.82	(0.63-1.06)	0.127	0.552	0.89	(0.7-1.12)	0.318	0.983	0.79	(0.47-1.34)	0.385	0.884
PC (diacyl) C36:5	ref.	0.90	(0.7-1.15)	0.390	0.823	0.88	(0.7-1.11)	0.288	0.983	0.71	(0.41-1.21)	0.203	0.760
PC (diacyl) C36:6	ref.	0.76	(0.58-0.99)	0.039	0.381	0.82	(0.65-1.05)	0.112	0.983	0.69	(0.41-1.18)	0.181	0.724
PC (diacyl) C38:0	ref.	1.03	(0.79-1.33)	0.845	0.973	0.98	(0.77-1.24)	0.866	0.983	0.96	(0.56-1.65)	0.881	0.960
PC (diacyl) C38:3	ref.	0.99	(0.76-1.3)	0.957	0.973	1.09	(0.85-1.4)	0.490	0.983	1.11	(0.62-1.99)	0.714	0.933
PC (diacyl) C38:4	ref.	0.91	(0.7-1.18)	0.475	0.823	0.90	(0.7-1.14)	0.380	0.983	0.91	(0.52-1.57)	0.724	0.933
PC (diacyl) C38:5	ref.	0.83	(0.64-1.08)	0.156	0.615	0.90	(0.71-1.14)	0.391	0.983	0.66	(0.38-1.14)	0.133	0.724
PC (diacyl) C38:6	ref.	0.92	(0.71-1.19)	0.538	0.823	0.97	(0.77-1.22)	0.791	0.983	0.91	(0.54-1.54)	0.726	0.933
PC (diacyl) C40:2	ref.	1.11	(0.86-1.45)	0.424	0.823	1.11	(0.87-1.42)	0.393	0.983	1.17	(0.69-2)	0.554	0.918
PC (diacyl) C40:3	ref.	1.01	(0.78-1.32)	0.932	0.973	1.06	(0.83-1.35)	0.632	0.983	1.44	(0.87-2.36)	0.154	0.724
PC (diacyl) C40:4	ref.	0.98	(0.76-1.26)	0.866	0.973	1.05	(0.83-1.32)	0.695	0.983	1.08	(0.63-1.84)	0.789	0.960
PC (diacyl) C40:5	ref.	0.99	(0.76-1.28)	0.934	0.973	1.15	(0.91-1.45)	0.257	0.983	1.02	(0.6-1.76)	0.930	0.960
PC (diacyl) C40:6	ref.	1.10	(0.84-1.42)	0.490	0.823	1.08	(0.85-1.37)	0.542	0.983	1.27	(0.74-2.17)	0.383	0.884
PC (diacyl) C42:0	ref.	0.96	(0.74-1.25)	0.756	0.963	1.01	(0.8-1.29)	0.912	0.983	0.88	(0.51-1.55)	0.667	0.932
PC (diacyl) C42:1	ref.	0.99	(0.76-1.29)	0.935	0.973	1.02	(0.8-1.3)	0.844	0.983	0.72	(0.42-1.26)	0.253	0.802
PC (diacyl) C42:2	ref.	0.90	(0.69-1.16)	0.411	0.823	0.94	(0.74-1.19)	0.610	0.983	0.79	(0.45-1.37)	0.396	0.884
PC (diacyl) C42:4	ref.	1.05	(0.82-1.36)	0.694	0.941	1.03	(0.82-1.3)	0.803	0.983	1.30	(0.77-2.21)	0.331	0.860
PC (diacyl) C42:5	ref.	1.02	(0.79-1.33)	0.874	0.973	1.16	(0.92-1.47)	0.206	0.983	1.18	(0.71-1.97)	0.521	0.888
PC (diacyl) C42:6	ref.	0.86	(0.67-1.1)	0.235	0.710	1.02	(0.8-1.3)	0.859	0.983	1.24	(0.66-2.32)	0.499	0.886
PC (acyl-alkyl) C30:0	ref.	0.84	(0.65-1.09)	0.188	0.671	1.07	(0.85-1.36)	0.549	0.983	1.31	(0.76-2.27)	0.328	0.860

PC (acyl-alkyl) C30:2	ref.	0.64	(0.48-0.84)	0.002	0.115	0.85	(0.66-1.1)	0.206	0.983	0.89	(0.49-1.62)	0.714	0.933
PC (acyl-alkyl) C32:1	ref.	0.91	(0.7-1.17)	0.449	0.823	0.98	(0.77-1.24)	0.839	0.983	1.06	(0.61-1.82)	0.842	0.960
PC (acyl-alkyl) C32:2	ref.	0.78	(0.6-1.02)	0.068	0.503	0.90	(0.7-1.15)	0.380	0.983	0.94	(0.54-1.64)	0.821	0.960
PC (acyl-alkyl) C34:0	ref.	0.94	(0.73-1.23)	0.660	0.922	1.20	(0.94-1.52)	0.139	0.983	1.45	(0.84-2.5)	0.184	0.724
PC (acyl-alkyl) C34:1	ref.	0.90	(0.68-1.17)	0.422	0.823	1.15	(0.89-1.47)	0.278	0.983	1.25	(0.7-2.23)	0.448	0.886
PC (acyl-alkyl) C34:2	ref.	0.92	(0.71-1.19)	0.532	0.823	0.92	(0.72-1.17)	0.507	0.983	0.80	(0.46-1.38)	0.415	0.884
PC (acyl-alkyl) C34:3	ref.	0.84	(0.65-1.1)	0.203	0.678	0.81	(0.63-1.03)	0.080	0.983	0.75	(0.43-1.3)	0.302	0.835
PC (acyl-alkyl) C36:0	ref.	1.17	(0.9-1.52)	0.241	0.711	1.20	(0.94-1.52)	0.137	0.983	1.44	(0.86-2.42)	0.169	0.724
PC (acyl-alkyl) C36:1	ref.	0.77	(0.59-1.01)	0.059	0.476	1.07	(0.84-1.37)	0.581	0.983	1.07	(0.61-1.89)	0.813	0.960
PC (acyl-alkyl) C36:2	ref.	0.80	(0.61-1.05)	0.113	0.552	0.99	(0.78-1.28)	0.964	0.983	0.97	(0.54-1.73)	0.916	0.960
PC (acyl-alkyl) C36:3	ref.	0.96	(0.74-1.25)	0.782	0.968	0.94	(0.74-1.19)	0.594	0.983	0.74	(0.43-1.27)	0.281	0.818
PC (acyl-alkyl) C36:4	ref.	0.96	(0.75-1.23)	0.730	0.963	0.91	(0.72-1.15)	0.426	0.983	0.83	(0.49-1.39)	0.473	0.886
PC (acyl-alkyl) C36:5	ref.	0.89	(0.69-1.14)	0.354	0.823	0.84	(0.66-1.06)	0.133	0.983	0.81	(0.48-1.38)	0.441	0.886
PC (acyl-alkyl) C38:0	ref.	0.81	(0.62-1.05)	0.114	0.552	0.83	(0.65-1.06)	0.130	0.983	0.69	(0.4-1.18)	0.174	0.724
PC (acyl-alkyl) C38:2	ref.	0.84	(0.64-1.11)	0.218	0.689	1.02	(0.79-1.31)	0.875	0.983	1.04	(0.57-1.87)	0.908	0.960
PC (acyl-alkyl) C38:3	ref.	0.81	(0.62-1.06)	0.127	0.552	1.13	(0.88-1.46)	0.323	0.983	0.91	(0.51-1.62)	0.754	0.933
PC (acyl-alkyl) C38:4	ref.	0.80	(0.62-1.03)	0.084	0.544	0.96	(0.76-1.22)	0.733	0.983	0.82	(0.48-1.39)	0.452	0.886
PC (acyl-alkyl) C38:5	ref.	0.95	(0.74-1.22)	0.672	0.930	0.86	(0.69-1.09)	0.217	0.983	0.77	(0.46-1.31)	0.340	0.868
PC (acyl-alkyl) C38:6	ref.	0.92	(0.71-1.19)	0.532	0.823	0.88	(0.7-1.12)	0.306	0.983	0.82	(0.48-1.39)	0.463	0.886
PC (acyl-alkyl) C40:1	ref.	0.84	(0.65-1.09)	0.192	0.671	0.82	(0.65-1.05)	0.112	0.983	0.57	(0.34-0.95)	0.032	0.724
PC (acyl-alkyl) C40:2	ref.	0.92	(0.71-1.19)	0.520	0.823	1.10	(0.87-1.39)	0.440	0.983	1.50	(0.87-2.56)	0.142	0.724
PC (acyl-alkyl) C40:3	ref.	0.83	(0.64-1.1)	0.195	0.671	0.96	(0.74-1.23)	0.735	0.983	0.87	(0.49-1.56)	0.645	0.932
PC (acyl-alkyl) C40:4	ref.	0.91	(0.7-1.17)	0.454	0.823	1.13	(0.89-1.43)	0.332	0.983	0.92	(0.53-1.57)	0.752	0.933

PC (acyl-alkyl) C40:5	ref.	0.80	(0.62-1.04)	0.098	0.552	1.03	(0.81-1.31)	0.785	0.983	0.83	(0.48-1.43)	0.504	0.886
PC (acyl-alkyl) C40:6	ref.	0.83	(0.64-1.08)	0.170	0.650	1.02	(0.8-1.3)	0.857	0.983	0.89	(0.52-1.53)	0.675	0.933
PC (acyl-alkyl) C42:1	ref.	0.91	(0.7-1.18)	0.464	0.823	0.88	(0.69-1.12)	0.297	0.983	0.68	(0.39-1.17)	0.163	0.724
PC (acyl-alkyl) C42:2	ref.	0.99	(0.76-1.29)	0.923	0.973	1.02	(0.8-1.3)	0.894	0.983	0.87	(0.49-1.52)	0.613	0.918
PC (acyl-alkyl) C42:3	ref.	0.90	(0.69-1.18)	0.457	0.823	0.91	(0.71-1.16)	0.450	0.983	0.59	(0.34-1.01)	0.053	0.724
PC (acyl-alkyl) C42:4	ref.	0.92	(0.71-1.2)	0.556	0.830	1.07	(0.84-1.36)	0.591	0.983	1.04	(0.6-1.82)	0.879	0.960
PC (acyl-alkyl) C42:5	ref.	0.85	(0.65-1.1)	0.209	0.678	1.01	(0.79-1.28)	0.941	0.983	0.73	(0.42-1.26)	0.252	0.802
PC (acyl-alkyl) C44:3	ref.	1.14	(0.87-1.51)	0.347	0.823	0.95	(0.75-1.19)	0.652	0.983	0.72	(0.49-1.06)	0.092	0.724
PC (acyl-alkyl) C44:4	ref.	0.93	(0.72-1.2)	0.553	0.830	1.07	(0.84-1.35)	0.598	0.983	0.84	(0.49-1.44)	0.526	0.888
PC (acyl-alkyl) C44:5	ref.	0.89	(0.69-1.15)	0.371	0.823	1.02	(0.8-1.28)	0.899	0.983	0.83	(0.49-1.41)	0.491	0.886
PC (acyl-alkyl) C44:6	ref.	0.89	(0.68-1.15)	0.363	0.823	0.91	(0.72-1.16)	0.447	0.983	0.87	(0.5-1.5)	0.610	0.918
SPHINGOLIPIDS													
Hydroxysphingomyelin C14:1	ref.	0.89	(0.68-1.15)	0.361	0.823	1.04	(0.81-1.33)	0.765	0.983	1.52	(0.8-2.9)	0.205	0.760
Hydroxysphingomyelin C16:1	ref.	0.82	(0.63-1.07)	0.145	0.604	0.88	(0.69-1.13)	0.321	0.983	0.88	(0.51-1.54)	0.665	0.932
Hydroxysphingomyelin C22:1	ref.	1.01	(0.78-1.31)	0.949	0.973	1.01	(0.79-1.28)	0.955	0.983	1.50	(0.87-2.6)	0.147	0.724
Hydroxysphingomyelin C22:2	ref.	0.71	(0.54-0.92)	0.011	0.245	0.73	(0.57-0.93)	0.013	0.983	1.17	(0.64-2.14)	0.615	0.918
Hydroxysphingomyelin C24:1	ref.	1.00	(0.78-1.29)	0.985	0.993	0.89	(0.71-1.12)	0.335	0.983	1.23	(0.75-2)	0.413	0.884
Sphingomyelin C16:0	ref.	0.74	(0.56-0.96)	0.026	0.336	1.00	(0.79-1.28)	0.975	0.983	1.26	(0.71-2.24)	0.430	0.886
Sphingomyelin C16:1	ref.	0.76	(0.58-1)	0.049	0.422	1.08	(0.84-1.38)	0.550	0.983	1.57	(0.91-2.73)	0.108	0.724
Sphingomyelin C18:0	ref.	0.74	(0.57-0.97)	0.029	0.341	0.93	(0.73-1.19)	0.586	0.983	0.97	(0.56-1.7)	0.924	0.960
Sphingomyelin C18:1	ref.	0.65	(0.49-0.86)	0.003	0.130	0.84	(0.65-1.08)	0.170	0.983	0.95	(0.53-1.71)	0.870	0.960
Sphingomyelin C20:2	ref.	0.76	(0.59-0.99)	0.041	0.381	0.87	(0.69-1.1)	0.248	0.983	1.26	(0.72-2.21)	0.409	0.884

Sphingomyelin C24:0	SM C24:0	ref.	0.95	(0.73-1.24)	0.728	0.963	0.99	(0.78-1.26)	0.920	0.983	1.51	(0.85-2.67)	0.159	0.724
Sphingomyelin C24:1	SM C24:1	ref.	0.72	(0.54-0.95)	0.019	0.278	0.75	(0.58-0.97)	0.029	0.983	0.97	(0.54-1.76)	0.928	0.960
Sphingomyelin C26:0	SM C26:0	ref.	0.90	(0.68-1.17)	0.422	0.823	1.12	(0.88-1.43)	0.370	0.983	1.62	(0.95-2.77)	0.077	0.724
Sphingomyelin C26:1	SM C26:1	ref.	0.71	(0.53-0.94)	0.019	0.278	0.91	(0.7-1.18)	0.463	0.983	1.18	(0.66-2.13)	0.574	0.918
HEXOSSES														
Hexoses	H1	ref.	1.12	(0.87-1.44)	0.395	0.823	1.08	(0.85-1.37)	0.527	0.983	0.51	(0.29-0.89)	0.017	0.724

COLON, ColoCare, CORSA (n=538)

Metabolite	Abbreviation	Stage I	Stage II			Stage III			Stage IV						
			OR ²	95% CI ³	p-value	p _{FDR} ⁴	OR ²	95% CI ³	p-value	p _{FDR} ⁴	OR ²	95% CI ³	p-value	p _{FDR} ⁴	
ACYLCARNITINES															
Carnitine	C0	ref.	1.14	(0.88-1.47)	0.313	0.997	0.94	(0.74-1.21)	0.644	0.790	0.74	(0.55-1)	0.048	0.273	
Acetyl carnitine	C2	ref.	0.94	(0.73-1.21)	0.629	0.997	0.90	(0.7-1.15)	0.383	0.580	0.81	(0.59-1.1)	0.184	0.490	
Propionyl carnitine	C3	ref.	1.11	(0.87-1.42)	0.411	0.997	0.98	(0.77-1.24)	0.852	0.930	0.91	(0.67-1.23)	0.534	0.788	
Butyryl carnitine	C4	ref.	1.13	(0.88-1.44)	0.341	0.997	1.06	(0.83-1.36)	0.622	0.770	0.85	(0.61-1.17)	0.322	0.627	
Valeryl carnitine	C5	ref.	0.87	(0.68-1.12)	0.273	0.997	0.96	(0.75-1.21)	0.714	0.836	0.99	(0.74-1.33)	0.940	0.966	
Dodecenoyl carnitine	C12:1	ref.	0.95	(0.74-1.22)	0.679	0.997	1.01	(0.79-1.3)	0.913	0.965	0.97	(0.71-1.32)	0.856	0.928	
Tetradecenoyl carnitine	C14:1	ref.	1.01	(0.79-1.3)	0.910	0.997	1.07	(0.84-1.36)	0.596	0.766	1.08	(0.79-1.46)	0.635	0.842	
Hexadecanoyl carnitine	C16	ref.	0.84	(0.66-1.08)	0.182	0.997	0.99	(0.78-1.26)	0.937	0.970	1.06	(0.79-1.42)	0.710	0.879	
Octadecanoyl carnitine	C18	ref.	0.88	(0.69-1.13)	0.328	0.997	0.96	(0.75-1.23)	0.747	0.867	1.04	(0.76-1.41)	0.825	0.925	
Octadecenoyl carnitine	C18:1	ref.	0.90	(0.7-1.15)	0.394	0.997	1.01	(0.8-1.28)	0.942	0.970	1.05	(0.78-1.41)	0.734	0.888	
Octadecadienyl carnitine	C18:2	ref.	0.92	(0.72-1.16)	0.467	0.997	0.88	(0.7-1.12)	0.303	0.525	0.87	(0.64-1.17)	0.346	0.643	
AMINO ACIDS															
Alanine	Ala	ref.	1.03	(0.8-1.31)	0.841	0.997	0.98	(0.77-1.25)	0.889	0.949	0.90	(0.66-1.22)	0.498	0.785	
Arginine	Arg	ref.	1.21	(0.94-1.55)	0.137	0.997	1.00	(0.79-1.25)	0.968	0.975	1.02	(0.76-1.37)	0.887	0.930	
Asparagine	Asn	ref.	1.02	(0.8-1.31)	0.848	0.997	0.92	(0.73-1.17)	0.511	0.697	0.90	(0.66-1.23)	0.513	0.785	
Aspartate	Asp	ref.	1.03	(0.81-1.31)	0.820	0.997	0.92	(0.73-1.17)	0.507	0.697	0.72	(0.53-0.99)	0.043	0.252	
Citrulline	Cit	ref.	1.00	(0.78-1.3)	0.974	0.997	0.86	(0.67-1.1)	0.233	0.445	0.55	(0.4-0.75)	0.000	0.017	
Glutamine	Gln	ref.	0.99	(0.77-1.26)	0.918	0.997	0.90	(0.71-1.14)	0.369	0.571	0.90	(0.66-1.22)	0.488	0.784	

Glutamate	Glu	ref.	0.94	(0.73-1.2)	0.606	0.997	0.98	(0.77-1.25)	0.889	0.949	1.24	(0.91-1.7)	0.168	0.489
Glycine	Gly	ref.	0.96	(0.75-1.23)	0.740	0.997	0.86	(0.67-1.09)	0.215	0.429	0.96	(0.7-1.31)	0.802	0.915
Histidine	His	ref.	0.83	(0.64-1.08)	0.160	0.997	0.83	(0.64-1.07)	0.156	0.344	0.68	(0.5-0.92)	0.014	0.138
Isoleucine	Ile	ref.	1.16	(0.91-1.48)	0.237	0.997	1.09	(0.86-1.38)	0.487	0.686	1.00	(0.74-1.36)	0.994	0.994
Leucine	Leu	ref.	0.96	(0.75-1.22)	0.719	0.997	0.98	(0.77-1.25)	0.891	0.949	0.83	(0.61-1.13)	0.235	0.537
Lysine	Lys	ref.	0.99	(0.78-1.27)	0.968	0.997	0.92	(0.72-1.17)	0.487	0.686	0.86	(0.63-1.16)	0.324	0.627
Methionine	Met	ref.	1.11	(0.86-1.42)	0.426	0.997	1.14	(0.9-1.45)	0.287	0.519	1.10	(0.81-1.49)	0.559	0.807
Ornithine	Orn	ref.	1.05	(0.83-1.35)	0.671	0.997	0.95	(0.75-1.2)	0.671	0.800	0.95	(0.71-1.29)	0.764	0.888
Phenylalanine	Phe	ref.	1.09	(0.86-1.38)	0.485	0.997	1.06	(0.84-1.34)	0.614	0.767	1.13	(0.83-1.55)	0.441	0.754
Proline	Pro	ref.	1.03	(0.81-1.31)	0.809	0.997	1.01	(0.8-1.28)	0.927	0.970	0.99	(0.73-1.35)	0.961	0.976
Serine	Ser	ref.	0.98	(0.77-1.25)	0.873	0.997	0.94	(0.74-1.19)	0.611	0.767	0.96	(0.7-1.3)	0.781	0.898
Threonine	Thr	ref.	1.05	(0.83-1.35)	0.671	0.997	0.93	(0.74-1.18)	0.574	0.754	1.03	(0.75-1.41)	0.856	0.928
Tryptophan	Trp	ref.	0.97	(0.75-1.25)	0.808	0.997	0.88	(0.69-1.12)	0.304	0.525	0.79	(0.58-1.08)	0.137	0.468
Tyrosine	Tyr	ref.	0.97	(0.76-1.23)	0.804	0.997	1.01	(0.79-1.28)	0.948	0.970	1.07	(0.78-1.46)	0.688	0.877
Valine	Val	ref.	0.90	(0.7-1.15)	0.394	0.997	0.94	(0.73-1.2)	0.603	0.767	0.82	(0.6-1.13)	0.225	0.537
BIOGENIC AMINES														
Creatinine	Creatinine	ref.	1.00	(0.77-1.31)	0.986	0.997	0.96	(0.74-1.26)	0.776	0.885	0.79	(0.56-1.12)	0.184	0.490
Kynurenine	Kynurenine	ref.	1.21	(0.94-1.55)	0.147	0.997	1.18	(0.92-1.51)	0.190	0.398	1.32	(0.96-1.81)	0.093	0.358
Sarcosine	Sarcosine	ref.	0.98	(0.77-1.25)	0.860	0.997	0.84	(0.66-1.07)	0.156	0.344	1.07	(0.8-1.44)	0.631	0.842
Trans 4-Hydroxyproline	t4-OH-Pro	ref.	1.17	(0.91-1.5)	0.217	0.997	1.13	(0.89-1.45)	0.314	0.525	1.17	(0.86-1.6)	0.319	0.627
Asymmetric dimethylarginine	ADMA	ref.	0.93	(0.73-1.2)	0.592	0.997	0.88	(0.69-1.13)	0.312	0.525	1.30	(0.96-1.76)	0.086	0.358
Symmetric dimethylarginine	SDMA	ref.	1.01	(0.79-1.29)	0.949	0.997	0.95	(0.74-1.21)	0.661	0.796	1.11	(0.8-1.53)	0.534	0.788

PC (diacyl) C36:2	ref.	1.05	(0.81-1.35)	0.708	0.997	0.83	(0.65-1.06)	0.132	0.312	0.95	(0.69-1.3)	0.734	0.888
PC (diacyl) C36:3	ref.	0.96	(0.74-1.23)	0.728	0.997	0.86	(0.67-1.1)	0.218	0.429	0.80	(0.59-1.09)	0.156	0.472
PC (diacyl) C36:4	ref.	0.81	(0.63-1.05)	0.112	0.997	0.79	(0.62-1.02)	0.073	0.202	0.68	(0.5-0.93)	0.017	0.139
PC (diacyl) C36:5	ref.	1.03	(0.81-1.32)	0.803	0.997	1.06	(0.83-1.34)	0.658	0.796	0.89	(0.66-1.21)	0.460	0.767
PC (diacyl) C36:6	ref.	0.93	(0.72-1.19)	0.556	0.997	0.86	(0.68-1.1)	0.245	0.454	0.76	(0.56-1.04)	0.084	0.358
PC (diacyl) C38:0	ref.	1.00	(0.77-1.3)	0.991	0.997	0.78	(0.6-1)	0.046	0.154	1.05	(0.76-1.44)	0.764	0.888
PC (diacyl) C38:3	ref.	0.94	(0.73-1.22)	0.660	0.997	0.88	(0.69-1.13)	0.329	0.535	0.89	(0.65-1.21)	0.449	0.757
PC (diacyl) C38:4	ref.	0.86	(0.66-1.12)	0.263	0.997	0.81	(0.62-1.04)	0.097	0.253	0.79	(0.57-1.08)	0.142	0.472
PC (diacyl) C38:5	ref.	0.93	(0.72-1.2)	0.554	0.997	0.93	(0.73-1.2)	0.590	0.766	0.76	(0.56-1.04)	0.086	0.358
PC (diacyl) C38:6	ref.	0.91	(0.71-1.17)	0.452	0.997	0.88	(0.69-1.13)	0.319	0.525	0.89	(0.66-1.22)	0.485	0.784
PC (diacyl) C40:2	ref.	1.21	(0.94-1.56)	0.132	0.997	0.89	(0.7-1.14)	0.368	0.571	1.04	(0.76-1.41)	0.816	0.922
PC (diacyl) C40:3	ref.	1.10	(0.86-1.42)	0.434	0.997	0.89	(0.7-1.14)	0.362	0.571	1.10	(0.81-1.5)	0.551	0.804
PC (diacyl) C40:4	ref.	0.91	(0.71-1.17)	0.458	0.997	0.90	(0.7-1.14)	0.376	0.576	0.82	(0.6-1.1)	0.189	0.490
PC (diacyl) C40:5	ref.	0.97	(0.76-1.25)	0.818	0.997	1.03	(0.81-1.32)	0.785	0.887	0.97	(0.71-1.31)	0.834	0.926
PC (diacyl) C40:6	ref.	1.05	(0.81-1.35)	0.731	0.997	1.01	(0.79-1.29)	0.961	0.975	1.14	(0.84-1.57)	0.403	0.698
PC (diacyl) C42:0	ref.	0.97	(0.75-1.25)	0.824	0.997	0.71	(0.55-0.92)	0.008	0.053	1.00	(0.73-1.37)	0.984	0.992
PC (diacyl) C42:1	ref.	0.98	(0.76-1.26)	0.856	0.997	0.68	(0.53-0.88)	0.003	0.026	0.86	(0.63-1.19)	0.360	0.650
PC (diacyl) C42:2	ref.	0.98	(0.76-1.28)	0.902	0.997	0.75	(0.58-0.97)	0.026	0.111	0.74	(0.54-1.02)	0.064	0.298
PC (diacyl) C42:4	ref.	0.98	(0.76-1.27)	0.891	0.997	0.73	(0.57-0.93)	0.012	0.066	0.95	(0.7-1.3)	0.745	0.888
PC (diacyl) C42:5	ref.	1.15	(0.9-1.47)	0.270	0.997	1.05	(0.82-1.34)	0.694	0.820	1.18	(0.87-1.59)	0.290	0.618
PC (diacyl) C42:6	ref.	0.89	(0.69-1.15)	0.381	0.997	0.88	(0.69-1.13)	0.317	0.525	0.86	(0.63-1.16)	0.324	0.627
PC (acyl-alkyl) C30:0	ref.	0.94	(0.73-1.2)	0.600	0.997	0.84	(0.66-1.08)	0.169	0.367	1.10	(0.81-1.51)	0.529	0.788
PC (acyl-alkyl) C30:2	ref.	0.80	(0.61-1.04)	0.093	0.997	0.72	(0.55-0.93)	0.013	0.066	0.78	(0.56-1.09)	0.149	0.472

PC (acyl-alkyl) C32:1	ref.	0.94	(0.73-1.21)	0.631	0.997	0.71	(0.56-0.92)	0.008	0.053	1.16	(0.84-1.59)	0.376	0.670
PC (acyl-alkyl) C32:2	ref.	0.90	(0.69-1.17)	0.443	0.997	0.71	(0.55-0.92)	0.011	0.063	1.02	(0.74-1.41)	0.909	0.945
PC (acyl-alkyl) C34:0	ref.	1.01	(0.78-1.3)	0.960	0.997	0.97	(0.76-1.25)	0.825	0.916	1.22	(0.89-1.67)	0.220	0.537
PC (acyl-alkyl) C34:1	ref.	1.00	(0.77-1.31)	0.997	0.997	0.90	(0.7-1.17)	0.436	0.637	1.37	(0.99-1.9)	0.061	0.298
PC (acyl-alkyl) C34:2	ref.	1.00	(0.78-1.3)	0.971	0.997	0.77	(0.6-0.99)	0.042	0.142	0.87	(0.64-1.19)	0.388	0.682
PC (acyl-alkyl) C34:3	ref.	0.93	(0.71-1.2)	0.561	0.997	0.69	(0.53-0.89)	0.004	0.030	0.78	(0.57-1.07)	0.121	0.426
PC (acyl-alkyl) C36:0	ref.	1.17	(0.91-1.5)	0.235	0.997	1.09	(0.85-1.39)	0.515	0.697	1.52	(1.12-2.07)	0.008	0.138
PC (acyl-alkyl) C36:1	ref.	0.96	(0.74-1.24)	0.738	0.997	0.91	(0.7-1.17)	0.464	0.670	1.01	(0.73-1.39)	0.944	0.966
PC (acyl-alkyl) C36:2	ref.	0.98	(0.76-1.28)	0.891	0.997	0.79	(0.61-1.02)	0.068	0.191	0.91	(0.66-1.26)	0.586	0.813
PC (acyl-alkyl) C36:3	ref.	1.02	(0.79-1.31)	0.906	0.997	0.77	(0.6-0.98)	0.036	0.130	0.83	(0.61-1.13)	0.240	0.538
PC (acyl-alkyl) C36:4	ref.	0.90	(0.7-1.16)	0.421	0.997	0.72	(0.56-0.92)	0.009	0.053	0.81	(0.6-1.1)	0.186	0.490
PC (acyl-alkyl) C36:5	ref.	0.85	(0.66-1.1)	0.217	0.997	0.67	(0.53-0.87)	0.002	0.026	0.80	(0.58-1.09)	0.154	0.472
PC (acyl-alkyl) C38:0	ref.	0.94	(0.73-1.21)	0.638	0.997	0.81	(0.63-1.04)	0.099	0.253	0.77	(0.56-1.05)	0.097	0.360
PC (acyl-alkyl) C38:2	ref.	1.01	(0.78-1.31)	0.911	0.997	0.80	(0.62-1.02)	0.075	0.202	0.94	(0.68-1.29)	0.704	0.879
PC (acyl-alkyl) C38:3	ref.	0.93	(0.72-1.21)	0.588	0.997	0.79	(0.61-1.01)	0.063	0.188	0.83	(0.6-1.13)	0.231	0.537
PC (acyl-alkyl) C38:4	ref.	0.83	(0.64-1.08)	0.169	0.997	0.68	(0.53-0.88)	0.003	0.026	0.81	(0.59-1.11)	0.184	0.490
PC (acyl-alkyl) C38:5	ref.	0.90	(0.69-1.16)	0.403	0.997	0.68	(0.53-0.88)	0.003	0.026	0.85	(0.62-1.16)	0.301	0.627
PC (acyl-alkyl) C38:6	ref.	0.92	(0.71-1.19)	0.531	0.997	0.74	(0.58-0.95)	0.020	0.089	0.85	(0.62-1.17)	0.328	0.627
PC (acyl-alkyl) C40:1	ref.	0.96	(0.74-1.26)	0.779	0.997	0.73	(0.57-0.94)	0.016	0.079	0.65	(0.47-0.88)	0.006	0.136
PC (acyl-alkyl) C40:2	ref.	1.03	(0.8-1.33)	0.807	0.997	0.88	(0.69-1.13)	0.318	0.525	1.27	(0.92-1.75)	0.148	0.472
PC (acyl-alkyl) C40:3	ref.	0.93	(0.72-1.22)	0.620	0.997	0.65	(0.5-0.85)	0.001	0.026	0.82	(0.59-1.14)	0.232	0.537
PC (acyl-alkyl) C40:4	ref.	0.90	(0.69-1.17)	0.420	0.997	0.67	(0.52-0.86)	0.002	0.026	0.86	(0.63-1.18)	0.353	0.647
PC (acyl-alkyl) C40:5	ref.	0.89	(0.68-1.16)	0.389	0.997	0.70	(0.54-0.9)	0.005	0.039	0.94	(0.68-1.29)	0.695	0.877

PC (acyl-alkyl) C40:6	ref.	0.89	(0.69-1.15)	0.380	0.997	0.76	(0.59-0.98)	0.036	0.130	0.97	(0.7-1.34)	0.848	0.928
PC (acyl-alkyl) C42:1	ref.	0.87	(0.66-1.15)	0.334	0.997	0.66	(0.5-0.86)	0.002	0.026	0.66	(0.48-0.91)	0.012	0.138
PC (acyl-alkyl) C42:2	ref.	0.99	(0.77-1.29)	0.955	0.997	0.75	(0.58-0.96)	0.023	0.100	0.90	(0.65-1.24)	0.511	0.785
PC (acyl-alkyl) C42:3	ref.	1.01	(0.78-1.32)	0.929	0.997	0.66	(0.51-0.86)	0.002	0.026	0.73	(0.53-1.01)	0.056	0.298
PC (acyl-alkyl) C42:4	ref.	0.94	(0.72-1.21)	0.611	0.997	0.67	(0.52-0.86)	0.002	0.026	0.93	(0.68-1.27)	0.628	0.842
PC (acyl-alkyl) C42:5	ref.	0.90	(0.7-1.16)	0.426	0.997	0.65	(0.5-0.83)	0.001	0.026	0.95	(0.7-1.3)	0.759	0.888
PC (acyl-alkyl) C44:3	ref.	1.24	(0.94-1.64)	0.131	0.997	0.83	(0.66-1.06)	0.132	0.312	0.92	(0.68-1.24)	0.574	0.813
PC (acyl-alkyl) C44:4	ref.	0.96	(0.75-1.24)	0.771	0.997	0.68	(0.53-0.88)	0.003	0.026	0.90	(0.66-1.23)	0.505	0.785
PC (acyl-alkyl) C44:5	ref.	0.96	(0.75-1.24)	0.751	0.997	0.67	(0.52-0.85)	0.001	0.026	0.93	(0.68-1.26)	0.642	0.843
PC (acyl-alkyl) C44:6	ref.	0.91	(0.7-1.17)	0.443	0.997	0.61	(0.47-0.78)	0.000	0.016	0.84	(0.61-1.15)	0.273	0.602
SPHINGOLIPIDS													
Hydroxysphingomyelin C14:1	ref.	0.82	(0.58-1.14)	0.239	0.997	0.70	(0.51-0.96)	0.029	0.114	1.03	(0.68-1.58)	0.885	0.930
Hydroxysphingomyelin C16:1	ref.	0.83	(0.64-1.06)	0.140	0.997	0.74	(0.57-0.95)	0.017	0.079	0.76	(0.55-1.05)	0.094	0.358
Hydroxysphingomyelin C22:1	ref.	1.06	(0.83-1.37)	0.636	0.997	0.89	(0.7-1.14)	0.347	0.557	1.48	(1.07-2.04)	0.018	0.141
Hydroxysphingomyelin C22:2	ref.	0.73	(0.57-0.95)	0.021	0.997	0.67	(0.52-0.87)	0.003	0.026	0.97	(0.7-1.36)	0.867	0.930
Hydroxysphingomyelin C24:1	ref.	1.06	(0.83-1.36)	0.631	0.997	0.93	(0.73-1.19)	0.571	0.754	1.42	(1.05-1.94)	0.025	0.176
Sphingomyelin C16:0	ref.	0.89	(0.68-1.16)	0.371	0.997	0.85	(0.65-1.1)	0.206	0.424	1.32	(0.94-1.85)	0.109	0.393
Sphingomyelin C16:1	ref.	0.91	(0.7-1.19)	0.493	0.997	0.91	(0.7-1.18)	0.491	0.686	1.58	(1.12-2.22)	0.009	0.138
Sphingomyelin C18:0	ref.	0.83	(0.64-1.08)	0.160	0.997	0.72	(0.56-0.93)	0.013	0.066	0.74	(0.54-1.02)	0.064	0.298
Sphingomyelin C18:1	ref.	0.80	(0.61-1.06)	0.116	0.997	0.67	(0.51-0.88)	0.003	0.027	0.73	(0.52-1.01)	0.058	0.298
Sphingomyelin C20:2	ref.	0.81	(0.63-1.05)	0.106	0.997	0.72	(0.56-0.93)	0.011	0.063	0.86	(0.62-1.17)	0.336	0.633
Sphingomyelin C24:0	ref.	0.96	(0.74-1.25)	0.751	0.997	0.80	(0.61-1.03)	0.080	0.213	1.47	(1.05-2.06)	0.026	0.176

Sphingomyelin C24:1	ref.	0.73	(0.56-0.96)	0.022	0.997	0.63	(0.48-0.82)	0.001	0.026	0.91	(0.65-1.28)	0.594	0.813
Sphingomyelin C26:0	ref.	0.94	(0.72-1.23)	0.644	0.997	1.00	(0.77-1.3)	0.995	0.995	1.72	(1.24-2.37)	0.001	0.047
Sphingomyelin C26:1	ref.	0.74	(0.56-0.98)	0.035	0.997	0.77	(0.58-1)	0.054	0.168	1.14	(0.81-1.6)	0.467	0.768
HEXOSSES													
Hexoses	ref.	1.10	(0.86-1.4)	0.440	0.997	1.19	(0.94-1.51)	0.153	0.344	1.02	(0.76-1.38)	0.884	0.930

Symmetric dimethylarginine	SDMA	ref.	1.09	(0.84-1.41)	0.504	0.965	0.98	(0.77-1.24)	0.859	0.980	1.13	(0.8-1.6)	0.492	0.770
GLYCEROPHOSPHOLIPIDS														
LysoPC (acyl) C16:0	lysoPC a C16:0	ref.	0.83	(0.64-1.07)	0.154	0.965	0.89	(0.7-1.14)	0.373	0.822	0.59	(0.43-0.81)	0.001	0.030
LysoPC (acyl) C16:1	lysoPC a C16:1	ref.	0.92	(0.72-1.19)	0.547	0.965	0.98	(0.77-1.24)	0.841	0.980	0.69	(0.51-0.93)	0.016	0.127
LysoPC (acyl) C17:0	lysoPC a C17:0	ref.	0.84	(0.65-1.1)	0.201	0.965	0.89	(0.69-1.13)	0.331	0.798	0.65	(0.47-0.88)	0.006	0.077
LysoPC (acyl) C18:0	lysoPC a C18:0	ref.	0.90	(0.69-1.17)	0.418	0.965	0.89	(0.7-1.14)	0.348	0.798	0.69	(0.51-0.95)	0.021	0.127
LysoPC (acyl) C18:1	lysoPC a C18:1	ref.	0.89	(0.68-1.16)	0.376	0.965	0.94	(0.74-1.21)	0.642	0.932	0.69	(0.51-0.94)	0.020	0.127
LysoPC (acyl) C18:2	lysoPC a C18:2	ref.	0.92	(0.7-1.2)	0.541	0.965	0.93	(0.73-1.19)	0.567	0.907	0.67	(0.49-0.92)	0.013	0.127
LysoPC (acyl) C20:3	lysoPC a C20:3	ref.	1.01	(0.78-1.31)	0.935	0.965	1.09	(0.86-1.39)	0.483	0.885	0.78	(0.58-1.06)	0.117	0.353
LysoPC (acyl) C20:4	lysoPC a C20:4	ref.	0.86	(0.67-1.11)	0.248	0.965	0.92	(0.72-1.16)	0.475	0.885	0.69	(0.5-0.94)	0.018	0.127
LysoPC (acyl) C28:1	lysoPC a C28:1	ref.	0.90	(0.69-1.16)	0.416	0.965	0.89	(0.7-1.13)	0.328	0.798	0.85	(0.62-1.18)	0.342	0.654
PC (diacyl) C28:1	PC aa C28:1	ref.	0.93	(0.72-1.2)	0.587	0.965	0.86	(0.68-1.1)	0.230	0.751	0.95	(0.69-1.31)	0.740	0.951
PC (diacyl) C30:0	PC aa C30:0	ref.	1.11	(0.86-1.43)	0.432	0.965	1.12	(0.89-1.42)	0.333	0.798	1.18	(0.86-1.61)	0.312	0.639
PC (diacyl) C32:0	PC aa C32:0	ref.	1.04	(0.81-1.35)	0.757	0.965	1.06	(0.84-1.35)	0.609	0.909	1.70	(1.22-2.36)	0.002	0.040
PC (diacyl) C32:1	PC aa C32:1	ref.	1.06	(0.82-1.37)	0.661	0.965	1.19	(0.94-1.51)	0.155	0.751	1.04	(0.76-1.43)	0.804	0.951
PC (diacyl) C32:2	PC aa C32:2	ref.	1.08	(0.84-1.4)	0.543	0.965	1.01	(0.8-1.29)	0.905	0.980	0.82	(0.6-1.13)	0.225	0.562
PC (diacyl) C32:3	PC aa C32:3	ref.	0.87	(0.67-1.14)	0.319	0.965	0.95	(0.74-1.22)	0.702	0.957	0.88	(0.63-1.24)	0.472	0.757
PC (diacyl) C34:1	PC aa C34:1	ref.	0.99	(0.76-1.28)	0.925	0.965	1.14	(0.9-1.45)	0.285	0.798	1.20	(0.87-1.65)	0.277	0.623
PC (diacyl) C34:2	PC aa C34:2	ref.	1.00	(0.78-1.29)	0.987	0.991	1.01	(0.8-1.28)	0.926	0.980	0.91	(0.66-1.25)	0.558	0.834
PC (diacyl) C34:3	PC aa C34:3	ref.	1.03	(0.79-1.34)	0.840	0.965	1.07	(0.84-1.37)	0.572	0.907	0.80	(0.58-1.1)	0.175	0.483
PC (diacyl) C34:4	PC aa C34:4	ref.	0.95	(0.73-1.23)	0.708	0.965	0.94	(0.74-1.19)	0.595	0.909	0.68	(0.49-0.92)	0.014	0.127
PC (diacyl) C36:0	PC aa C36:0	ref.	0.94	(0.73-1.22)	0.656	0.965	0.90	(0.71-1.14)	0.399	0.850	0.97	(0.7-1.33)	0.834	0.951

PC (diacyl) C36:1	ref.	1.08	(0.83-1.4)	0.580	0.965	1.06	(0.83-1.34)	0.656	0.938	1.08	(0.78-1.48)	0.656	0.901
PC (diacyl) C36:2	ref.	1.10	(0.85-1.42)	0.477	0.965	0.99	(0.78-1.25)	0.916	0.980	0.97	(0.7-1.35)	0.870	0.956
PC (diacyl) C36:3	ref.	1.05	(0.81-1.36)	0.693	0.965	1.04	(0.82-1.32)	0.738	0.957	0.86	(0.62-1.17)	0.329	0.639
PC (diacyl) C36:4	ref.	0.79	(0.61-1.03)	0.081	0.965	0.86	(0.68-1.1)	0.231	0.751	0.68	(0.49-0.95)	0.022	0.127
PC (diacyl) C36:5	ref.	0.81	(0.63-1.04)	0.094	0.965	0.93	(0.74-1.17)	0.512	0.907	0.74	(0.54-1.02)	0.066	0.251
PC (diacyl) C36:6	ref.	0.90	(0.69-1.16)	0.398	0.965	0.92	(0.72-1.16)	0.480	0.885	0.74	(0.54-1.01)	0.061	0.251
PC (diacyl) C38:0	ref.	0.99	(0.77-1.28)	0.942	0.965	0.88	(0.69-1.11)	0.272	0.798	1.13	(0.82-1.57)	0.447	0.747
PC (diacyl) C38:3	ref.	1.13	(0.87-1.46)	0.370	0.965	1.07	(0.84-1.36)	0.603	0.909	1.03	(0.74-1.41)	0.880	0.956
PC (diacyl) C38:4	ref.	0.87	(0.67-1.13)	0.305	0.965	0.86	(0.67-1.1)	0.221	0.751	0.83	(0.6-1.16)	0.276	0.623
PC (diacyl) C38:5	ref.	0.78	(0.61-1.02)	0.067	0.965	0.89	(0.7-1.14)	0.363	0.813	0.68	(0.49-0.94)	0.018	0.127
PC (diacyl) C38:6	ref.	0.90	(0.7-1.16)	0.411	0.965	0.96	(0.76-1.22)	0.744	0.957	0.87	(0.63-1.2)	0.411	0.731
PC (diacyl) C40:2	ref.	1.08	(0.84-1.39)	0.552	0.965	0.94	(0.74-1.19)	0.596	0.909	0.96	(0.7-1.33)	0.822	0.951
PC (diacyl) C40:3	ref.	0.95	(0.74-1.23)	0.716	0.965	0.93	(0.73-1.17)	0.525	0.907	1.00	(0.73-1.38)	0.995	0.995
PC (diacyl) C40:4	ref.	1.04	(0.8-1.33)	0.788	0.965	1.01	(0.8-1.28)	0.908	0.980	0.89	(0.65-1.22)	0.485	0.769
PC (diacyl) C40:5	ref.	0.98	(0.76-1.26)	0.861	0.965	1.08	(0.86-1.37)	0.506	0.907	0.96	(0.7-1.31)	0.802	0.951
PC (diacyl) C40:6	ref.	1.07	(0.83-1.38)	0.590	0.965	1.06	(0.84-1.35)	0.615	0.909	1.16	(0.84-1.6)	0.366	0.684
PC (diacyl) C42:0	ref.	1.07	(0.82-1.38)	0.630	0.965	0.88	(0.69-1.11)	0.277	0.798	1.10	(0.79-1.52)	0.585	0.865
PC (diacyl) C42:1	ref.	1.10	(0.85-1.42)	0.471	0.965	0.85	(0.67-1.08)	0.180	0.751	0.98	(0.71-1.36)	0.908	0.959
PC (diacyl) C42:2	ref.	0.98	(0.75-1.29)	0.901	0.965	0.82	(0.64-1.04)	0.105	0.697	0.72	(0.52-0.99)	0.045	0.217
PC (diacyl) C42:4	ref.	1.08	(0.84-1.41)	0.538	0.965	0.81	(0.64-1.03)	0.081	0.697	0.96	(0.7-1.32)	0.802	0.951
PC (diacyl) C42:5	ref.	0.96	(0.75-1.23)	0.735	0.965	0.94	(0.75-1.19)	0.613	0.909	1.04	(0.76-1.42)	0.813	0.951
PC (diacyl) C42:6	ref.	0.86	(0.66-1.12)	0.261	0.965	0.91	(0.71-1.16)	0.429	0.880	0.75	(0.55-1.02)	0.065	0.251
PC (acyl-alkyl) C30:0	ref.	1.02	(0.79-1.31)	0.882	0.965	0.97	(0.76-1.23)	0.788	0.980	1.19	(0.87-1.64)	0.272	0.623

PC (acyl-alkyl) C30:2	ref.	0.82	(0.63-1.07)	0.148	0.965	0.80	(0.62-1.02)	0.075	0.697	0.83	(0.59-1.16)	0.278	0.623
PC (acyl-alkyl) C32:1	ref.	0.97	(0.75-1.25)	0.798	0.965	0.88	(0.7-1.12)	0.302	0.798	1.29	(0.93-1.79)	0.132	0.380
PC (acyl-alkyl) C32:2	ref.	0.81	(0.62-1.06)	0.125	0.965	0.82	(0.64-1.05)	0.117	0.697	1.04	(0.74-1.45)	0.820	0.951
PC (acyl-alkyl) C34:0	ref.	1.06	(0.82-1.37)	0.654	0.965	1.05	(0.83-1.34)	0.664	0.938	1.23	(0.89-1.69)	0.212	0.552
PC (acyl-alkyl) C34:1	ref.	0.98	(0.76-1.28)	0.895	0.965	1.00	(0.79-1.28)	0.969	0.980	1.35	(0.97-1.88)	0.079	0.284
PC (acyl-alkyl) C34:2	ref.	0.97	(0.75-1.26)	0.838	0.965	0.91	(0.71-1.15)	0.433	0.880	0.88	(0.64-1.2)	0.416	0.731
PC (acyl-alkyl) C34:3	ref.	0.90	(0.7-1.17)	0.448	0.965	0.84	(0.66-1.07)	0.158	0.751	0.80	(0.58-1.1)	0.167	0.471
PC (acyl-alkyl) C36:0	ref.	1.13	(0.87-1.46)	0.365	0.965	1.13	(0.89-1.43)	0.333	0.798	1.58	(1.15-2.18)	0.005	0.077
PC (acyl-alkyl) C36:1	ref.	0.96	(0.74-1.24)	0.753	0.965	1.00	(0.79-1.28)	0.972	0.980	1.01	(0.73-1.4)	0.963	0.991
PC (acyl-alkyl) C36:2	ref.	1.01	(0.77-1.31)	0.957	0.972	0.97	(0.76-1.24)	0.817	0.980	0.93	(0.67-1.28)	0.647	0.901
PC (acyl-alkyl) C36:3	ref.	1.02	(0.79-1.31)	0.908	0.965	0.93	(0.73-1.18)	0.555	0.907	0.87	(0.64-1.19)	0.389	0.711
PC (acyl-alkyl) C36:4	ref.	0.94	(0.73-1.21)	0.631	0.965	0.86	(0.68-1.08)	0.197	0.751	0.90	(0.66-1.23)	0.512	0.790
PC (acyl-alkyl) C36:5	ref.	0.83	(0.65-1.08)	0.165	0.965	0.80	(0.63-1.01)	0.062	0.697	0.88	(0.64-1.21)	0.439	0.747
PC (acyl-alkyl) C38:0	ref.	0.88	(0.68-1.14)	0.329	0.965	0.89	(0.7-1.13)	0.350	0.798	0.74	(0.53-1.02)	0.066	0.251
PC (acyl-alkyl) C38:2	ref.	1.13	(0.87-1.46)	0.372	0.965	0.98	(0.77-1.24)	0.848	0.980	1.00	(0.72-1.37)	0.984	0.992
PC (acyl-alkyl) C38:3	ref.	1.07	(0.82-1.38)	0.622	0.965	0.98	(0.77-1.24)	0.860	0.980	0.93	(0.67-1.27)	0.631	0.892
PC (acyl-alkyl) C38:4	ref.	0.95	(0.74-1.23)	0.720	0.965	0.85	(0.67-1.08)	0.193	0.751	0.94	(0.69-1.3)	0.721	0.951
PC (acyl-alkyl) C38:5	ref.	0.90	(0.69-1.16)	0.412	0.965	0.79	(0.62-1)	0.053	0.697	0.93	(0.68-1.29)	0.668	0.904
PC (acyl-alkyl) C38:6	ref.	0.86	(0.67-1.12)	0.258	0.965	0.83	(0.65-1.05)	0.117	0.697	0.89	(0.64-1.22)	0.459	0.747
PC (acyl-alkyl) C40:1	ref.	0.89	(0.68-1.17)	0.394	0.965	0.78	(0.61-1.01)	0.058	0.697	0.61	(0.44-0.83)	0.002	0.044
PC (acyl-alkyl) C40:2	ref.	1.09	(0.85-1.42)	0.494	0.965	0.96	(0.75-1.21)	0.711	0.957	1.32	(0.95-1.83)	0.099	0.314
PC (acyl-alkyl) C40:3	ref.	1.07	(0.82-1.4)	0.605	0.965	0.82	(0.64-1.04)	0.105	0.697	0.93	(0.67-1.29)	0.658	0.901

PC (acyl-alkyl) C40:4	ref.	1.09	(0.84-1.41)	0.499	0.965	0.89	(0.7-1.12)	0.316	0.798	1.04	(0.76-1.43)	0.798	0.951
PC (acyl-alkyl) C40:5	ref.	0.97	(0.75-1.25)	0.795	0.965	0.85	(0.67-1.08)	0.187	0.751	1.05	(0.75-1.45)	0.786	0.951
PC (acyl-alkyl) C40:6	ref.	0.95	(0.74-1.23)	0.719	0.965	0.91	(0.72-1.15)	0.433	0.880	1.05	(0.76-1.45)	0.784	0.951
PC (acyl-alkyl) C42:1	ref.	1.01	(0.77-1.33)	0.943	0.965	0.82	(0.64-1.05)	0.124	0.700	0.75	(0.55-1.02)	0.068	0.251
PC (acyl-alkyl) C42:2	ref.	1.07	(0.82-1.38)	0.627	0.965	0.86	(0.68-1.1)	0.224	0.751	0.91	(0.65-1.25)	0.549	0.830
PC (acyl-alkyl) C42:3	ref.	1.03	(0.79-1.35)	0.809	0.965	0.77	(0.6-0.99)	0.039	0.697	0.75	(0.54-1.04)	0.081	0.284
PC (acyl-alkyl) C42:4	ref.	1.07	(0.82-1.38)	0.627	0.965	0.84	(0.66-1.06)	0.144	0.746	1.02	(0.74-1.41)	0.891	0.956
PC (acyl-alkyl) C42:5	ref.	1.02	(0.79-1.32)	0.892	0.965	0.84	(0.66-1.06)	0.137	0.742	1.11	(0.81-1.53)	0.517	0.790
PC (acyl-alkyl) C44:3	ref.	1.23	(0.94-1.6)	0.127	0.965	0.98	(0.78-1.24)	0.897	0.980	0.93	(0.68-1.25)	0.621	0.888
PC (acyl-alkyl) C44:4	ref.	1.06	(0.82-1.37)	0.662	0.965	0.87	(0.68-1.1)	0.239	0.759	1.02	(0.74-1.4)	0.914	0.959
PC (acyl-alkyl) C44:5	ref.	1.04	(0.8-1.34)	0.777	0.965	0.83	(0.66-1.05)	0.118	0.697	1.06	(0.77-1.45)	0.734	0.951
PC (acyl-alkyl) C44:6	ref.	0.99	(0.77-1.28)	0.940	0.965	0.79	(0.62-1)	0.047	0.697	0.95	(0.69-1.31)	0.746	0.951
SPHINGOLIPIDS													
Hydroxy sphingomyelin C14:1	ref.	0.97	(0.73-1.28)	0.815	0.965	0.91	(0.71-1.17)	0.480	0.885	1.30	(0.87-1.93)	0.196	0.521
Hydroxy sphingomyelin C16:1	ref.	0.95	(0.73-1.24)	0.713	0.965	0.86	(0.68-1.1)	0.225	0.751	0.80	(0.58-1.11)	0.185	0.501
Hydroxy sphingomyelin C22:1	ref.	1.12	(0.87-1.45)	0.384	0.965	1.02	(0.8-1.28)	0.899	0.980	1.56	(1.11-2.18)	0.010	0.116
Hydroxy sphingomyelin C22:2	ref.	0.82	(0.63-1.07)	0.145	0.965	0.72	(0.57-0.92)	0.009	0.697	0.97	(0.7-1.35)	0.865	0.956
Hydroxy sphingomyelin C24:1	ref.	1.04	(0.81-1.34)	0.753	0.965	0.89	(0.7-1.13)	0.341	0.798	1.45	(1.05-1.99)	0.022	0.127
Sphingomyelin C16:0	ref.	0.93	(0.71-1.21)	0.592	0.965	0.91	(0.71-1.16)	0.458	0.885	1.34	(0.95-1.88)	0.092	0.301
Sphingomyelin C16:1	ref.	0.95	(0.72-1.23)	0.681	0.965	0.98	(0.77-1.26)	0.892	0.980	1.68	(1.18-2.39)	0.004	0.070
Sphingomyelin C18:0	ref.	0.94	(0.72-1.22)	0.621	0.965	0.80	(0.62-1.02)	0.069	0.697	0.76	(0.55-1.05)	0.093	0.301
Sphingomyelin C18:1	ref.	0.85	(0.65-1.12)	0.242	0.965	0.77	(0.6-0.99)	0.040	0.697	0.77	(0.55-1.07)	0.115	0.353

Spingomyelin C20:2	SM C20:2	ref.	0.91	(0.7-1.18)	0.474	0.965	0.79	(0.62-1)	0.055	0.697	0.83	(0.61-1.15)	0.270	0.623
Spingomyelin C24:0	SM C24:0	ref.	1.09	(0.84-1.41)	0.517	0.965	0.97	(0.76-1.23)	0.804	0.980	1.54	(1.09-2.17)	0.014	0.127
Spingomyelin C24:1	SM C24:1	ref.	0.81	(0.62-1.06)	0.128	0.965	0.77	(0.6-1)	0.047	0.697	0.99	(0.69-1.4)	0.933	0.970
Spingomyelin C26:0	SM C26:0	ref.	0.98	(0.75-1.29)	0.906	0.965	1.10	(0.85-1.41)	0.475	0.885	1.99	(1.42-2.8)	0.000	0.009
Spingomyelin C26:1	SM C26:1	ref.	0.76	(0.57-1.01)	0.059	0.965	0.87	(0.67-1.13)	0.309	0.798	1.32	(0.93-1.89)	0.120	0.354
HEXOSES														
Hexoses	HI	ref.	1.05	(0.82-1.35)	0.677	0.965	1.15	(0.91-1.45)	0.249	0.771	0.98	(0.72-1.34)	0.890	0.956

Bold metabolites are significantly associated with colorectal cancer stage after FDR correction in the total study population; **Bold** p-values are significant associations in individual Leave-one-out analyses;¹ Analysed using multinomial logistic regression models analysing associations of colorectal cancer stage as independent variable (stage I is reference) and the residuals obtained from linear mixed models with log transformed Z-standardized metabolite concentrations as dependent variable with random intercepts for analytical batch nested within cohort. Regression models were adjusted for sex, age and body mass index (continuous);² untransformed and unadjusted metabolite concentrations in µmol/l; ³Odds Ratio (eß), for stage II, III and IV vs. I-II colorectal cancer, respectively, per SD increase in transformed metabolite concentrations; ⁴Confidence Interval; ⁵p-value corrected for False Discovery Rate.

Supplementary Table S6 Results of plasma metabolites associated with colorectal cancer stage (III-IV vs. I-II)¹ in the total study population

Metabolite	Abbreviation	Stage I-II ² Mean ± SD	Stage III-IV ² Mean ± SD	OR ³	95% CI ⁴	p-value	p _{FDR} ⁵
ACYLCARNITINES							
Carnitine	C0	37.30 ± 8.76	36.33 ± 9.39	0.88	(0.75-1.02)	0.090	0.378
Acetyl carnitine	C2	7.03 ± 3.11	6.79 ± 2.73	0.96	(0.82-1.12)	0.613	0.781
Propionyl carnitine	C3	0.43 ± 0.15	0.42 ± 0.15	0.97	(0.84-1.13)	0.732	0.840
Butyryl carnitine	C4	0.24 ± 0.16	0.22 ± 0.09	0.97	(0.84-1.13)	0.701	0.836
Valeryl carnitine	C5	0.18 ± 0.10	0.19 ± 0.15	1.09	(0.94-1.27)	0.266	0.571
Dodecenoyl carnitine	C12:1	0.14 ± 0.06	0.14 ± 0.06	1.03	(0.89-1.21)	0.659	0.809
Tetradecenoyl carnitine	C14:1	0.07 ± 0.04	0.06 ± 0.04	1.06	(0.92-1.24)	0.419	0.682
Hexadecanoyl carnitine	C16	0.12 ± 0.04	0.12 ± 0.04	1.14	(0.98-1.32)	0.093	0.378
Octadecanoyl carnitine	C18	0.05 ± 0.02	0.05 ± 0.02	1.09	(0.94-1.26)	0.272	0.571
Octadecenoyl carnitine	C18:1	0.11 ± 0.07	0.12 ± 0.06	1.06	(0.91-1.23)	0.437	0.686
Octadecadienyl carnitine	C18:2	0.04 ± 0.03	0.04 ± 0.03	0.92	(0.79-1.06)	0.251	0.571
AMINO ACIDS							
Alanine	Ala	367.02 ± 109.45	364.18 ± 107.80	0.99	(0.85-1.15)	0.918	0.955
Arginine	Arg	69.96 ± 27.59	65.81 ± 27.77	0.91	(0.79-1.06)	0.238	0.571
Asparagine	Asn	43.18 ± 10.94	42.30 ± 10.40	0.89	(0.76-1.04)	0.133	0.401
Aspartate	Asp	9.84 ± 4.13	9.15 ± 4.00	0.93	(0.80-1.08)	0.320	0.581
Citrulline	Cit	32.98 ± 11.40	30.46 ± 11.23	0.84	(0.72-0.98)	0.027	0.275
Glutamine	Gln	687.62 ± 126.99	675.58 ± 110.76	0.92	(0.79-1.07)	0.292	0.581
Glutamate	Glu	71.20 ± 32.68	72.72 ± 34.41	1.14	(0.98-1.33)	0.091	0.378
Glycine	Gly	214.66 ± 63.20	215.97 ± 60.5	0.99	(0.85-1.15)	0.903	0.947
Histidine	His	70.99 ± 15.25	70.55 ± 15.75	0.91	(0.78-1.05)	0.203	0.539
Isoleucine	Ile	76.12 ± 24.94	75.16 ± 23.39	0.95	(0.81-1.10)	0.475	0.690
Leucine	Leu	130.35 ± 41.70	128.45 ± 39.34	0.93	(0.80-1.08)	0.365	0.613
Lysine	Lys	194.93 ± 51.64	189.46 ± 47.79	0.87	(0.75-1.01)	0.077	0.378
Methionine	Met	19.56 ± 6.43	19.56 ± 6.06	1.04	(0.89-1.21)	0.618	0.781
Ornithine	Orn	79.07 ± 29.72	79.69 ± 28.55	0.92	(0.79-1.07)	0.267	0.571
Phenylalanine	Phe	61.52 ± 15.09	62.02 ± 20.41	1.02	(0.88-1.18)	0.817	0.900
Proline	Pro	219.04 ± 81.25	213.76 ± 80.90	0.98	(0.85-1.14)	0.838	0.908
Serine	Ser	94.11 ± 24.01	94.94 ± 23.04	1.01	(0.87-1.17)	0.947	0.967
Threonine	Thr	109.35 ± 30.36	108.51 ± 31.46	0.93	(0.80-1.08)	0.326	0.581

Metabolite	Abbreviation	Stage I-II ² Mean ± SD	Stage III-IV ² Mean ± SD	OR ³	95% CI ⁴	p-value	p _{DR} ⁵
Tryptophan	Trp	52.04 ± 14.04	50.99 ± 14.73	0.89	(0.76-1.03)	0.123	0.401
Tyrosine	Tyr	68.93 ± 22.10	66.73 ± 19.60	0.97	(0.83-1.12)	0.659	0.809
Valine	Val	230.72 ± 62.13	228.70 ± 59.51	0.95	(0.82-1.11)	0.515	0.720
BIOGENIC AMINES							
Creatinine	Creatinine	79.82 ± 22.48	79.31 ± 28.39	0.94	(0.80-1.12)	0.511	0.720
Kynurenine	Kynurenine	2.69 ± 0.91	2.68 ± 0.92	1.06	(0.91-1.24)	0.427	0.685
Sarcosine	Sarcosine	4.19 ± 2.31	4.05 ± 2.64	1.01	(0.87-1.17)	0.901	0.947
Trans 4-Hydroxyproline	t4-OH-Pro	10.09 ± 7.24	9.93 ± 5.57	1.06	(0.91-1.23)	0.471	0.690
Asymmetric dimethylarginine	ADMA	0.47 ± 0.11	0.48 ± 0.11	1.02	(0.88-1.19)	0.754	0.852
Symmetric dimethylarginine	SDMA	0.50 ± 0.17	0.49 ± 0.17	0.98	(0.84-1.14)	0.779	0.865
GLYCEROPHOSPHOLIPIDS							
Lysophosphatidylcholine (acyl) C16:0	lysoPC a C16:0	93.23 ± 26.93	92.77 ± 27.66	0.92	(0.80-1.07)	0.305	0.581
Lysophosphatidylcholine (acyl) C16:1	lysoPC a C16:1	2.54 ± 0.93	2.54 ± 1.02	0.95	(0.82-1.10)	0.460	0.690
Lysophosphatidylcholine (acyl) C17:0	lysoPC a C17:0	1.56 ± 0.57	1.56 ± 0.62	0.93	(0.80-1.08)	0.345	0.591
Lysophosphatidylcholine (acyl) C18:0	lysoPC a C18:0	26.35 ± 8.59	25.75 ± 9.05	0.89	(0.77-1.04)	0.131	0.401
Lysophosphatidylcholine (acyl) C18:1	lysoPC a C18:1	18.62 ± 6.22	18.76 ± 6.72	0.93	(0.80-1.08)	0.323	0.581
Lysophosphatidylcholine (acyl) C18:2	lysoPC a C18:2	27.36 ± 11.34	26.71 ± 12.7	0.86	(0.74-1.00)	0.058	0.378
Lysophosphatidylcholine (acyl) C20:3	lysoPC a C20:3	2.02 ± 0.76	2.07 ± 0.85	0.97	(0.84-1.13)	0.723	0.840
Lysophosphatidylcholine (acyl) C20:4	lysoPC a C20:4	6.12 ± 2.15	5.94 ± 2.08	0.91	(0.78-1.06)	0.214	0.557
Lysophosphatidylcholine (acyl) C28:1	lysoPC a C28:1	0.24 ± 0.07	0.23 ± 0.08	0.97	(0.83-1.14)	0.714	0.840
Phosphatidylcholine (diacyl) C28:1	PC aa C28:1	2.78 ± 0.74	2.70 ± 0.76	0.93	(0.80-1.09)	0.385	0.634
Phosphatidylcholine (diacyl) C30:0	PC aa C30:0	3.76 ± 1.30	3.90 ± 1.52	1.09	(0.93-1.26)	0.287	0.581
Phosphatidylcholine (diacyl) C32:0	PC aa C32:0	25.16 ± 5.69	25.44 ± 6.42	1.12	(0.97-1.31)	0.132	0.401
Phosphatidylcholine (diacyl) C32:1	PC aa C32:1	29.42 ± 15.44	30.43 ± 16.15	1.10	(0.94-1.27)	0.224	0.561
Phosphatidylcholine (diacyl) C32:2	PC aa C32:2	6.98 ± 3.17	6.75 ± 3.47	0.92	(0.79-1.07)	0.281	0.580
Phosphatidylcholine (diacyl) C32:3	PC aa C32:3	0.77 ± 0.25	0.75 ± 0.28	0.96	(0.81-1.12)	0.587	0.763
Phosphatidylcholine (diacyl) C34:1	PC aa C34:1	345.18 ± 80.91	353.26 ± 83.23	1.13	(0.97-1.31)	0.120	0.401
Phosphatidylcholine (diacyl) C34:2	PC aa C34:2	624.62 ± 139.26	603.39 ± 134.80	0.95	(0.82-1.11)	0.540	0.747
Phosphatidylcholine (diacyl) C34:3	PC aa C34:3	25.56 ± 10.69	24.68 ± 10.91	0.95	(0.81-1.10)	0.475	0.690
Phosphatidylcholine (diacyl) C34:4	PC aa C34:4	2.73 ± 1.18	2.60 ± 1.28	0.89	(0.76-1.04)	0.130	0.401
Phosphatidylcholine (diacyl) C36:0	PC aa C36:0	2.89 ± 0.94	2.76 ± 0.94	0.92	(0.79-1.07)	0.272	0.571
Phosphatidylcholine (diacyl) C36:1	PC aa C36:1	58.97 ± 18.48	59.22 ± 19.79	1.00	(0.86-1.17)	0.960	0.967

Metabolite	Abbreviation	Stage I-II ² Mean ± SD	Stage III-IV ² Mean ± SD	OR ³	95% CI ⁴	p-value	p _{DR} ⁵
Phosphatidyletholine (diacyl) C36:2	PC aa C36:2	330.49 ± 88.36	317.58 ± 94.41	0.90	(0.77-1.05)	0.195	0.539
Phosphatidyletholine (diacyl) C36:3	PC aa C36:3	178.13 ± 45.07	177.21 ± 51.73	0.96	(0.82-1.11)	0.562	0.763
Phosphatidyletholine (diacyl) C36:4	PC aa C36:4	280.39 ± 69.11	270.47 ± 67.51	0.92	(0.79-1.08)	0.306	0.581
Phosphatidyletholine (diacyl) C36:5	PC aa C36:5	38.67 ± 23.51	37.15 ± 21.35	0.97	(0.84-1.13)	0.722	0.840
Phosphatidyletholine (diacyl) C36:6	PC aa C36:6	1.28 ± 0.63	1.22 ± 0.64	0.92	(0.79-1.07)	0.255	0.571
Phosphatidyletholine (diacyl) C38:0	PC aa C38:0	2.65 ± 0.79	2.54 ± 0.85	0.91	(0.78-1.06)	0.221	0.561
Phosphatidyletholine (diacyl) C38:3	PC aa C38:3	52.11 ± 15.04	52.27 ± 17.02	0.99	(0.85-1.15)	0.882	0.947
Phosphatidyletholine (diacyl) C38:4	PC aa C38:4	131.30 ± 34.43	126.46 ± 35.94	0.91	(0.78-1.07)	0.256	0.571
Phosphatidyletholine (diacyl) C38:5	PC aa C38:5	61.70 ± 17.99	60.32 ± 18.65	0.96	(0.82-1.12)	0.586	0.763
Phosphatidyletholine (diacyl) C38:6	PC aa C38:6	92.58 ± 30.28	89.73 ± 29.26	0.98	(0.85-1.14)	0.827	0.904
Phosphatidyletholine (diacyl) C40:2	PC aa C40:2	0.25 ± 0.09	0.24 ± 0.09	0.90	(0.78-1.05)	0.197	0.539
Phosphatidyletholine (diacyl) C40:3	PC aa C40:3	0.37 ± 0.13	0.37 ± 0.12	0.96	(0.83-1.12)	0.608	0.781
Phosphatidyletholine (diacyl) C40:4	PC aa C40:4	2.98 ± 0.82	3.00 ± 0.99	0.97	(0.84-1.13)	0.737	0.840
Phosphatidyletholine (diacyl) C40:5	PC aa C40:5	7.76 ± 2.28	7.94 ± 2.73	1.08	(0.93-1.26)	0.315	0.581
Phosphatidyletholine (diacyl) C40:6	PC aa C40:6	25.79 ± 8.71	25.37 ± 9.03	1.03	(0.89-1.20)	0.679	0.818
Phosphatidyletholine (diacyl) C42:0	PC aa C42:0	0.32 ± 0.10	0.31 ± 0.10	0.89	(0.76-1.03)	0.125	0.401
Phosphatidyletholine (diacyl) C42:1	PC aa C42:1	0.16 ± 0.05	0.15 ± 0.05	0.84	(0.72-0.98)	0.025	0.269
Phosphatidyletholine (diacyl) C42:2	PC aa C42:2	0.14 ± 0.04	0.13 ± 0.04	0.83	(0.71-0.97)	0.016	0.269
Phosphatidyletholine (diacyl) C42:4	PC aa C42:4	0.10 ± 0.02	0.10 ± 0.03	0.84	(0.72-0.98)	0.025	0.269
Phosphatidyletholine (diacyl) C42:5	PC aa C42:5	0.23 ± 0.08	0.23 ± 0.08	1.03	(0.89-1.20)	0.671	0.816
Phosphatidyletholine (diacyl) C42:6	PC aa C42:6	0.32 ± 0.09	0.32 ± 0.10	0.99	(0.86-1.15)	0.927	0.956
Phosphatidyletholine (acyl-alkyl) C30:0	PC ae C30:0	0.31 ± 0.09	0.31 ± 0.11	1.02	(0.88-1.19)	0.768	0.861
Phosphatidyletholine (acyl-alkyl) C30:2	PC ae C30:2	0.08 ± 0.02	0.07 ± 0.02	0.92	(0.79-1.09)	0.340	0.589
Phosphatidyletholine (acyl-alkyl) C32:1	PC ae C32:1	4.78 ± 1.23	4.64 ± 1.33	0.94	(0.81-1.10)	0.444	0.686
Phosphatidyletholine (acyl-alkyl) C32:2	PC ae C32:2	1.19 ± 0.35	1.13 ± 0.34	0.94	(0.8-1.10)	0.441	0.686
Phosphatidyletholine (acyl-alkyl) C34:0	PC ae C34:0	2.19 ± 0.62	2.20 ± 0.73	1.09	(0.94-1.27)	0.270	0.571
Phosphatidyletholine (acyl-alkyl) C34:1	PC ae C34:1	16.58 ± 3.84	16.72 ± 4.33	1.09	(0.93-1.28)	0.296	0.581
Phosphatidyletholine (acyl-alkyl) C34:2	PC ae C34:2	18.61 ± 5.65	17.90 ± 6.20	0.88	(0.76-1.03)	0.118	0.401
Phosphatidyletholine (acyl-alkyl) C34:3	PC ae C34:3	11.99 ± 4.12	11.27 ± 4.14	0.83	(0.72-0.97)	0.021	0.269
Phosphatidyletholine (acyl-alkyl) C36:0	PC ae C36:0	1.04 ± 0.30	1.06 ± 0.35	1.10	(0.95-1.28)	0.200	0.539
Phosphatidyletholine (acyl-alkyl) C36:1	PC ae C36:1	10.76 ± 2.66	10.82 ± 3.00	1.05	(0.89-1.22)	0.581	0.763
Phosphatidyletholine (acyl-alkyl) C36:2	PC ae C36:2	19.34 ± 5.54	18.92 ± 6.00	0.95	(0.81-1.11)	0.502	0.717
Phosphatidyletholine (acyl-alkyl) C36:3	PC ae C36:3	10.51 ± 3.06	10.11 ± 3.32	0.87	(0.75-1.01)	0.075	0.378
Phosphatidyletholine (acyl-alkyl) C36:4	PC ae C36:4	25.01 ± 6.84	23.78 ± 7.04	0.87	(0.75-1.01)	0.060	0.378

Metabolite	Abbreviation	Stage I-II ² Mean ± SD	Stage III-IV ² Mean ± SD	OR ³	95% CI ⁴	p-value	p _{DR} ⁵
Phosphatidylcholine (acyl-alkyl) C36:5	PC ae C36:5	16.84 ± 4.83	15.82 ± 4.99	0.86	(0.74-0.99)	0.041	0.378
Phosphatidylcholine (acyl-alkyl) C38:0	PC ae C38:0	2.16 ± 0.80	2.04 ± 0.80	0.89	(0.76-1.03)	0.121	0.401
Phosphatidylcholine (acyl-alkyl) C38:2	PC ae C38:2	2.04 ± 0.60	2.01 ± 0.71	0.93	(0.79-1.08)	0.326	0.581
Phosphatidylcholine (acyl-alkyl) C38:3	PC ae C38:3	4.50 ± 1.12	4.46 ± 1.28	0.96	(0.82-1.12)	0.572	0.763
Phosphatidylcholine (acyl-alkyl) C38:4	PC ae C38:4	16.34 ± 3.61	15.71 ± 4.03	0.89	(0.77-1.04)	0.145	0.418
Phosphatidylcholine (acyl-alkyl) C38:5	PC ae C38:5	21.64 ± 4.98	20.63 ± 5.34	0.84	(0.72-0.97)	0.019	0.269
Phosphatidylcholine (acyl-alkyl) C38:6	PC ae C38:6	8.52 ± 2.47	8.12 ± 2.58	0.88	(0.76-1.02)	0.098	0.387
Phosphatidylcholine (acyl-alkyl) C40:1	PC ae C40:1	1.03 ± 0.30	0.96 ± 0.33	0.79	(0.68-0.93)	0.003	0.177
Phosphatidylcholine (acyl-alkyl) C40:2	PC ae C40:2	1.53 ± 0.38	1.54 ± 0.45	1.00	(0.86-1.16)	0.972	0.972
Phosphatidylcholine (acyl-alkyl) C40:3	PC ae C40:3	0.83 ± 0.19	0.80 ± 0.21	0.82	(0.70-0.97)	0.017	0.269
Phosphatidylcholine (acyl-alkyl) C40:4	PC ae C40:4	2.13 ± 0.47	2.06 ± 0.52	0.89	(0.76-1.03)	0.130	0.401
Phosphatidylcholine (acyl-alkyl) C40:5	PC ae C40:5	3.32 ± 0.70	3.27 ± 0.78	0.92	(0.79-1.07)	0.267	0.571
Phosphatidylcholine (acyl-alkyl) C40:6	PC ae C40:6	4.43 ± 1.15	4.33 ± 1.26	0.96	(0.83-1.12)	0.629	0.787
Phosphatidylcholine (acyl-alkyl) C42:1	PC ae C42:1	0.30 ± 0.06	0.29 ± 0.07	0.79	(0.68-0.93)	0.004	0.177
Phosphatidylcholine (acyl-alkyl) C42:2	PC ae C42:2	0.40 ± 0.10	0.39 ± 0.12	0.86	(0.73-1.00)	0.054	0.378
Phosphatidylcholine (acyl-alkyl) C42:3	PC ae C42:3	0.50 ± 0.14	0.47 ± 0.16	0.76	(0.65-0.89)	0.0008	0.099
Phosphatidylcholine (acyl-alkyl) C42:4	PC ae C42:4	0.65 ± 0.18	0.63 ± 0.19	0.86	(0.74-1.00)	0.058	0.378
Phosphatidylcholine (acyl-alkyl) C42:5	PC ae C42:5	1.55 ± 0.35	1.52 ± 0.39	0.88	(0.75-1.02)	0.089	0.378
Phosphatidylcholine (acyl-alkyl) C44:3	PC ae C44:3	0.06 ± 0.02	0.06 ± 0.02	0.83	(0.71-0.97)	0.020	0.269
Phosphatidylcholine (acyl-alkyl) C44:4	PC ae C44:4	0.22 ± 0.06	0.21 ± 0.07	0.87	(0.74-1.01)	0.066	0.378
Phosphatidylcholine (acyl-alkyl) C44:5	PC ae C44:5	1.01 ± 0.30	0.98 ± 0.31	0.86	(0.74-1.00)	0.044	0.378
Phosphatidylcholine (acyl-alkyl) C44:6	PC ae C44:6	0.76 ± 0.22	0.72 ± 0.22	0.81	(0.69-0.94)	0.007	0.231
SPHINGOLIPIDS							
Hydroxysphingomyelin C14:1	SM OH C14:1	4.10 ± 1.12	4.15 ± 1.20	1.06	(0.91-1.24)	0.448	0.686
Hydroxysphingomyelin C16:1	SM OH C16:1	1.94 ± 0.53	2.02 ± 0.62	1.15	(0.98-1.35)	0.089	0.378
Hydroxysphingomyelin C22:1	SM OH C22:1	4.65 ± 1.20	4.57 ± 1.35	0.88	(0.75-1.02)	0.093	0.378
Hydroxysphingomyelin C22:2	SM OH C22:2	3.75 ± 1.04	3.68 ± 1.08	0.87	(0.74-1.02)	0.081	0.378
Hydroxysphingomyelin C24:1	SM OH C24:1	0.35 ± 0.10	0.34 ± 0.11	0.88	(0.75-1.02)	0.088	0.378
Sphingomyelin C16:0	SM C16:0	71.39 ± 13.87	72.07 ± 16.34	0.99	(0.85-1.16)	0.893	0.947
Sphingomyelin C16:1	SM C16:1	10.07 ± 2.37	9.76 ± 2.33	0.88	(0.75-1.04)	0.137	0.404
Sphingomyelin C18:0	SM C18:0	13.55 ± 3.78	14.44 ± 4.74	1.21	(1.03-1.42)	0.018	0.269
Sphingomyelin C18:1	SM C18:1	6.34 ± 1.90	6.44 ± 1.96	1.08	(0.91-1.27)	0.368	0.613
Sphingomyelin C20:2	SM C20:2	0.15 ± 0.06	0.15 ± 0.07	0.96	(0.82-1.11)	0.565	0.763

Metabolite	Abbreviation	Stage I-II ² Mean ± SD	Stage III-IV ² Mean ± SD	OR ³	95% CI ⁴	p-value	p _{FDR} ⁵
Sphingomyelin C24:0	SM C24:0	6.72 ± 1.77	6.71 ± 1.88	0.87	(0.75-1.02)	0.079	0.378
Sphingomyelin C24:1	SM C24:1	15.40 ± 3.91	15.72 ± 4.42	1.00	(0.86-1.16)	0.953	0.967
Sphingomyelin C26:0	SM C26:0	0.04 ± 0.01	0.04 ± 0.01	0.87	(0.75-1.01)	0.064	0.378
Sphingomyelin C26:1	SM C26:1	0.11 ± 0.04	0.11 ± 0.04	0.95	(0.82-1.10)	0.478	0.690
HEXOSSES							
Hexoses	HI	5275.14 ± 1581.78	5241.75 ± 1703.27	1.08	(0.93-1.25)	0.340	0.589

²Analysed using multiple logistic regression models analysing associations of colorectal cancer stage (III-IV vs. I-II) as outcome and as the exposure the residuals obtained from linear mixed models with log transformed Z-standardized metabolite concentrations as dependent variable with random intercepts for analytical batch nested within cohort. Regression models were adjusted for sex, age and body mass index (continuous); ³untransformed and unadjusted metabolite concentrations in µmol/l; ³Odds Ratio (eß), for stage III-IV vs. I-II colorectal cancer per SD increase in transformed metabolite concentrations; ⁴Confidence Interval; ⁵p-value corrected for False Discovery Rate.

*A nutritional
perspective*

4

Chapter 4

Diet and metabolites in colorectal cancer patients

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Abstract

Introduction Emerging evidence suggests that diet is linked to survival in colorectal cancer patients, although underlying mechanisms are not fully understood. The aim of this study was to evaluate whether dietary exposures are associated with metabolite concentrations in colorectal cancer patients.

Methods Concentrations of 134 metabolites of the Biocrates Absolute^{IDQ} p180 kit were quantified in plasma samples collected at diagnosis from 195 stage I-IV colorectal cancer patients. Food frequency questionnaires were used to calculate adherence to the World Cancer Research Fund (WCRF) dietary recommendations and the Dutch Healthy Diet (DHD15) index as well as to construct dietary patterns using Principal Component Analysis. Multivariable linear regression models were used to determine associations between dietary exposures and metabolite concentrations. All models were adjusted for age, sex, body mass index, smoking status, analytical batch, cancer stage, and multiple testing using False Discovery Rate.

Results Participants had a mean (SD) age of 66 (9) years, were mostly men (60%), and mostly diagnosed with stage II and III cancer. For the dietary pattern analyses, Western, Carnivore, and Prudent patterns were identified. Better adherence to the WCRF dietary recommendations was associated with lower concentrations of ten phosphatidylcholines. Higher intake of the Carnivore pattern was associated with higher concentrations of two phosphatidylcholines. The DHD15-index, Western pattern, or Prudent pattern were not associated with metabolite concentrations.

Conclusion In the current study, the WCRF dietary score and the Carnivore pattern are associated with phosphatidylcholines. Future research should elucidate the potential relevance of phosphatidylcholine metabolism in the colorectal cancer continuum.

Key words: Colorectal cancer patients, diet quality indices, dietary patterns, metabolite, metabolomics

Introduction

Previous research suggests that there is a relation between diet and survival after colorectal cancer diagnosis [1]. However, the underlying mechanisms are largely unknown. The identification of metabolites associated with diet in colorectal cancer patients could be a first step in unravelling the link between dietary exposures and colorectal cancer progression [2-4] and understanding the biological processes involved [5, 6]. Metabolomics measures a range of small molecules, many of which belong to a number of different biochemical pathways. Such metabolic profiles can provide a snapshot of the current metabolic state of the body, characteristic of a phenotype [7] and are, therefore, increasingly used to study the interface of diet, lifestyle and diseases [8-10].

Thus far, research has shown that dietary exposures can be associated with metabolite concentrations in blood. Predefined diet quality indices [11] as well as distinct dietary patterns such as veganism [12, 13] were reported to be associated with specific blood metabolites. Three diet quality indices were associated with metabolites, including mainly lipids and amino acids [11]. Another study reported that a vegan diet was associated with lower concentrations of glycerophospholipids, sphingolipids and amino acids compared to a diet containing meat and/or fish [12].

In terms of understanding the complex relationship between diet and metabolites, investigating dietary patterns and indices, instead of single nutrients or food groups, is of specific interest since nutrients and food groups may interact with each other [14]. Diet quality indices and dietary patterns are used to assess exposure to combinations of food groups. An example of a diet quality index, commonly used to investigate the relationship with health outcomes after cancer diagnosis [15-17], is the World Cancer Research Fund (WCRF) score that is based on the 2018 cancer prevention recommendations of the WCRF/American Institute for Cancer Research (AICR) [18]. The Dutch Healthy Diet index (DHD15-index) is used to assess the adherence to the Dutch dietary guidelines of 2015 [19] and has also previously been associated with various health outcomes [20, 21]. In addition to these indices, dietary patterns are data-driven and are also commonly evaluated against health outcomes after cancer diagnosis [22].

It is well-established that disease can have a great impact on metabolism [23-25] and, to the best of our knowledge, no research has been conducted investigating the associations between dietary exposures and metabolites in cancer patients. Therefore, the

aim of this study was to investigate whether the diet, evaluated using diet quality indices and dietary patterns, is associated with plasma metabolites in colorectal cancer patients.

Methods

Study population

In total, 200 stage I-IV colorectal cancer patients with available plasma metabolite concentrations of the COLON study [26], a prospective cohort study among colorectal cancer patients in the Netherlands, were considered for the present study. The design and recruitment of the COLON study has been described earlier [26]. Participants were recruited from eleven hospitals in the Netherlands, shortly after colorectal cancer diagnosis. Females and males of all ages and of any stage of the disease were eligible. Non-Dutch speaking patients, patients with a history of colorectal cancer or (partial) bowel resection, chronic inflammatory bowel disease, hereditary colorectal cancer syndromes (e.g. Lynch syndrome, Familial Adenomatous Polyposis, Peutz-Jegher), dementia or another mental condition causing an inability to fill out a questionnaire correctly were excluded. All participants provided a written informed consent. The COLON study was approved by the Committee on Research involving Human Subjects (region Arnhem-Nijmegen), the Netherlands.

Participants with missing dietary intake data ($n=2$) or with a missing cancer stage ($n=3$) were excluded from the current study, resulting in a final study population of $n=195$ stage I-IV colorectal cancer patients for analysis.

Data collection

Habitual dietary intake in the month prior to diagnosis was assessed using a 204-item validated, semi-quantitative food frequency questionnaire (FFQ) developed by the Division of Human Nutrition and Health of Wageningen University & Research, the Netherlands [27, 28]. The FFQ was used to calculate *a priori* defined diet quality indices and to construct *a posteriori* data-driven dietary patterns. Demographic and lifestyle characteristics such as sex, age, weight, height, and smoking habits were assessed using self-administered questionnaires. All questionnaires were filled out prior to tumor resection. Medical information, including cancer stage, tumor location, and treatment strategies, was collected using the Dutch ColoRectal Audit [29].

Non-fasted plasma EDTA samples were collected upon recruitment, which were

intended before the start of treatment, and stored at -80°C using a standardized protocol to ensure identical sample handling across the eleven hospitals.

Diet quality indices

Two diet quality indices have been included in the current study, namely the WCRF dietary score and the DHD15-index. Briefly, the WCRF dietary score is based on the 2018 WCRF/AICR recommendations for cancer prevention using the standard WCRF/AICR score developed by Shams-White *et al.* [18]. Since the current study focusses on dietary intake, the recommendations regarding weight, physical activity, supplement use, and breastfeeding were not included. The remaining recommendations were: 1) eat a diet rich in whole grains, vegetables, fruits, and beans, 2) limit consumption of ‘fast foods’ and other processed foods high in fat, starches or sugar, 3) limit consumption of red and processed meat, 4) limit consumption of sugar-sweetened drinks, and 5) reduce alcohol consumption. Quantitative criteria were used as cut-off points for all recommendations, except for the recommendation 2) limit consumption of ‘fast foods’ and other processed foods high in fat, starches or sugar, where cut-offs were based on tertiles calculated as a percentage of total energy intake from processed foods. Processed foods included French fries, crisps, pastry and biscuits, savory snacks, sugar and candy, sauces, pizza, pancake, sandwich fillings high in sugar or fat, refined grain products, and sweet dairy desserts. Not included were yoghurt and cheese, nuts, oils and fats, sugary drinks, processed meat, and diet soft drinks. Sugary drinks included sugar-sweetened soft drinks, sugar-sweetened dairy drinks, and fruit juices.

The score assigned for each recommendation was 1 when the recommendation was met (full adherence), a score of 0.5 was assigned to moderate adherence and a score of 0 was assigned to low adherence. The recommendation regarding a diet rich in whole grains, vegetables, fruit, and beans, included sub-recommendations for fiber intake and for fruit and vegetable consumption. As a result, the recommendation score was the sum of sub-recommendation scores of fiber intake and fruit and vegetables intake, meaning that plausible scores were 0, 0.25, 0.5, 0.75, and 1. The overall score of the WCRF dietary score ranged from 0 to 5.

The DHD15-index [19] was developed on the basis of the 2015 Dutch dietary guidelines [30] and refers to 15 recommendations. In the current study, the recommendations regarding coffee consumption and sodium intake were excluded since the type of coffee and sodium intake were not assessed in the COLON study. The DHD15-

index used in the current study included the intake of sugary drinks, liquid fats and oils, processed meat, red meat, nuts, dairy, refined grains products, whole grain products, vegetables, alcohol, legumes, solid fat, fruit, fish, and tea. The scores for each individual recommendation ranged from 0 to 10 points with a maximum total score of 130 points.

For both indices, a higher score represents a healthier diet, i.e. a better compliance with the recommendations of the corresponding diet quality index. Details on the used diet quality indices have been described before [18, 19, 31].

Empirical construction of dietary patterns

Total intake of food items (g/d) and total energy intake (kcal/d) were calculated based on frequency of intake, number of portions, portion size, and the type of products, as recorded in the FFQ. All food items were categorized into 33 food groups, that were constructed according to the Dutch food composition table 2011 [32]. Final food groups are described in **Supplementary Table S1**. Total intake of food groups was recalculated to relative intake (g/d per 1000kcal) using total energy intake to simplify comparison of participants.

Principal component analysis was used to investigate data-driven dietary patterns among participants [33]. Food group data were log-transformed using the natural logarithm and Z-standardized before performing principal component analysis. As a result, the intake of all food groups has a mean of zero and a variance of one, which is important since the results of components highly depend on the variance of each variable [34]. In case a certain food group was not consumed, i.e. 0 g/day per 1000kcal, 0.001 was added to the food group sum to allow log transformation. The number of dietary patterns was decided based on the components with eigenvalues >1.0 , the scree plot and the interpretability of the components [35]. The remaining components were orthogonally rotated for ease of interpretation and labels in accordance with the included food groups were given. Positive and negative food group loadings $>|0.2|$ were considered to contribute to the dietary pattern. Participants' scores were determined by multiplying the observed intake of food groups by the factor loading for each of the relevant food groups [36].

Three dietary patterns were identified based on the available data, which were defined as a Western, Carnivore, and Prudent dietary pattern, see **Figure 1**. The Western dietary pattern was characterized by a high intake of snacks, savory sauces and spreads, refined grains, pizza, high and medium-fat dairy, nuts and seeds, beer, and hard fats, and a

low intake of whole grain products and potatoes. The Carnivore pattern was characterized by a high intake of red and processed meat, poultry, fish, eggs, and potatoes, and a low intake of soy and vegetarian products, and medium and high-fat dairy. Lastly, the Prudent pattern consisted of a high intake of vegetables, fruits, fish, nuts and seeds, low-fat dairy, tea, pastry and biscuits, and a low intake of beer.

Biomarker analysis

Plasma samples were analyzed in four analytical batches at the International Agency for Research on Cancer (IARC) in Lyon, France. In total, 147 metabolites were measured using the Absolute^{IDQ} p180 kit (Biocrates, Innsbruck, Austria). The analytical method [37, 38] characterizes up to 188 metabolites from five compound classes. Amino acids and biogenic amines were quantified (calibration curves, individual isotope-labelled internal standards) by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Lipids, including glycerophospholipids and sphingolipids, acylcarnitines, as well as the sum of hexose sugars were semi-quantified (one-point calibration, single representative internal standard) by flow injection analysis-tandem mass spectrometry (FIA-MS/MS).

Figure 1 Overview of food group loadings of the Western, Carnivore, and Prudent dietary patterns. Green and red bars represent positive and negative loading strengths, respectively. A more positive loading illustrates higher consumption of a specific food group, while a more negative loading characterizes lower consumption of the food group. Only food group loadings $>|0.2|$ were considered to contribute to the dietary pattern and visualized to improve readability.

Metabolites with >20% missing values (n=13), including values below the level of detection (LOD) and true missings, were removed from the dataset while remaining missing values were imputed in line with previous studies [38-40]. Briefly, missing values below the LOD were imputed by half of the batch-specific LOD and values below or above the quantitative range were replaced by the lower or upper limit of quantification, respectively. Subsequently, to normalize distributions, metabolite concentrations were log transformed using the natural logarithm and were Z-standardized to allow comparison of estimates among metabolites. In total, 134 metabolites were included in the current analysis (**Supplementary Table S2**), consisting of 12 acylcarnitines (Cx:y), 21 amino acids, 8 biogenic amines, 78 glycerophospholipids (10 lysophosphatidylcholines (lysoPC) and 68 phosphatidylcholines (PC diacyl (aa) and acyl-alkyl (ae)), 14 sphingolipids (SM Cx:y) and the sum of hexoses. The abbreviation Cx:y is used to describe the total number of carbons and double bonds in the alkyl chains, respectively.

Statistical analysis

Clinical, demographic and lifestyle characteristics were described using descriptive analyses. Linear regression models were used to determine the associations between diet quality indices and dietary patterns as independent variables and concentrations of metabolites as dependent variables. Diet quality indices and dietary patterns were analyzed as continuous variables as well as analyzed in tertiles, for which the lowest tertile, corresponding to the lowest intake of the exposure, was used as the reference category. *P*-trend values were computed for tertiles using the medians of the corresponding tertiles. The continuous analysis is presented per one-point increase for the WCRF dietary score, per ten-points increase for the DHD15-index and per each increase in standard deviation (SD) for the Western, Carnivore, and Prudent pattern.

All models were adjusted for age at diagnosis (continuous), sex, BMI (continuous), smoking status (current smoker/former smoker/never smoker), analytical batch (1-4), and cancer stage (stage I/stage II/ stage III/ stage IV). The basis for assessing whether covariates should be included in the final model were existing evidence, biological plausibility and whether the regression coefficient of interest changed by >10% after adding the potential covariate.

Furthermore, to explore the consistency in the observed associations between dietary exposures and plasma metabolites, we evaluated the top-15 metabolites (based on the

smallest p -value for trend over tertiles) associated with diet quality indices and dietary patterns using a heatmap.

Sensitivity analyses were conducted excluding patients from whom blood was collected during or after any type of treatment, i.e. (neo-) adjuvant chemotherapy and/or surgery ($n=19$) and excluding stage IV patients ($n=7$).

All statistical analyses were performed in R, version 3.4.0 and SAS, version 9.4. After correction for multiple testing, using false discovery rate (FDR) according to the Benjamin-Hochberg procedure [41, 42], a p -value (p_{FDR}) <0.05 was considered statistically significant.

Results

Study population characteristics

Characteristics of the overall study population are summarized in **Table 1**. The mean (SD) age of the 195 colorectal cancer patients was 66 (9) years and almost 60% of the study population was male. Mean (SD) BMI was 25.6 (4.9) kg/m^2 , around ten percent were current smokers and participants had a stage I (27%), stage II (33%), stage III (36%) or stage IV (4%) cancer. Distal colon cancer was diagnosed in 36% of the study population, proximal colon cancer and rectal cancer in 29% and 35%, respectively. The study population had a mean (SD) total energy intake of 1856 (559) kcal/day. The mean (SD) WCRF dietary score and DHD15-index were 2.1 (0.7) and 73.8 (14.1), respectively. Baseline characteristics of the participants in the lowest and highest tertile of each diet quality index and dietary pattern are shown in **Supplementary Table S3**.

Diet quality indices

Table 2 presents the top-15 metabolites based on the smallest value of p_{trend} across tertiles for the analyses between diet quality indices and metabolite concentrations, which were ranked by the p_{trend} across tertiles of the diet quality indices. A higher concordance of the WCRF dietary score was statistically significantly associated after FDR adjustment with lower concentrations of ten phosphatidylcholines over increasing tertiles. Each one-point increase in the WCRF dietary score also showed statistically significantly lower concentrations of four of the above-mentioned ten phosphatidylcholines (PC ae C36:3, PC ae C36:4, PC aa C36:3, and PC aa C38:3).

The DHD15-index was not statistically significantly associated with plasma metabolites after FDR adjustment when analyzed by tertiles of the DHD15-index and continuously (**Table 2**). An overview of all the results on the association between the diet quality indices and all 134 metabolites is available in **Supplementary Table S2**.

Dietary patterns

The top-15 metabolites resulting from analysis of associations between the Western, Carnivore, and Prudent pattern and plasma metabolites, ranked by the p_{trend} across tertiles of the dietary patterns, are shown in **Table 3**. No linear trend was observed over increasing tertiles of the Western pattern in relation to plasma metabolites. In contrast, every SD increase in consumption of the Western pattern was statistically significantly associated with 35 metabolites (**Table 3** and **Supplementary Table S2**).

A linear trend was observed between increasing tertiles of the Carnivore pattern and higher concentrations of two phosphatidylcholines. Similarly, every SD increase in the intake of the Carnivore pattern was also statistically significantly associated with higher concentrations of PC aa C38:0 (p_{FDR} : 0.001) and PC ae 38:6 (p_{FDR} : 0.01).

The Prudent pattern was not statistically significantly associated with any metabolite when evaluating the linear trend, as well as when testing each SD increase in consumption of the Prudent pattern. Results of the analyses between the dietary patterns and all 134 metabolites are provided in **Supplementary Table S2**.

Overlap in the top-15 metabolites

Figure 2 illustrates the overlap in the observed top-15 metabolites (based on the smallest p -value for trend over tertiles) for each of the respective diet quality indices and dietary patterns. No overlap among acylcarnitines in the top-15 metabolites for each of the dietary exposures was observed. One amino acid was overlapping; the Carnivore and Prudent pattern both showed a positive association with plasma tryptophan. Sarcosine, a biogenic amine, was positively associated with both the DHD15-index and the Prudent pattern.

Table 1 Baseline characteristics of the overall study population

		<i>Study population</i>
Number of participants		195
Male sex, n (%)		115 (59)
Age		66.2 ± 9.1
Body mass index (kg/m ²)		25.6 ± 4.9
Underweight, <18.5, n (%)		3 (2)
Normal weight, 18.5-24.9, n (%)		85 (44)
Overweight, 25-29.9, n (%)		79 (41)
Obese, ≥30, n (%)		28 (14)
Smoking, n (%)		
Current		19 (10)
Former		118 (60)
Never		58 (30)
Stage, n (%)		
I		53 (27)
II		65 (33)
III		70 (36)
IV		7 (4)
Tumor site ¹ , n (%)		
Colon – proximal		56 (29)
Colon – distal		71 (36)
Rectal		68 (35)
Treatment, n (%)		
Surgery		194 (99)
Neo-adjuvant treatment		60 (31)
Total energy intake (kcal/d)		1856 ± 559
Total WCRF dietary score ²		2.1 ± 0.7
Total DHD15-index ³		73.8 ± 14.1

Numbers are presented as mean ± SD or median (IQR) unless mentioned otherwise;

¹Proximal consisting of: hepatic flexure, transverse colon, cecum, appendix, ascending colon; Distal consisting of: descending colon, sigmoid colon, splenic flexure; Rectal consisting of: rectosigmoid junction, rectum; ²Adherence to the dietary recommendations of the World Cancer Research Fund (WCRF), ranged 0 to 5 [18]; ³Adherence to the Dutch Healthy Diet guidelines 2015 (DHD15), ranged 0 to 130 [19].

Table 2 Top-15 plasma metabolites associated with diet quality indices, ranked by p_{trend}

Continuous			Tertiles		
			1	2	3
Diet quality index: WCRF dietary score^{1,2}					
No. of participants			77	56	62
Mean score (range)			1.40 (0.50 to 1.75)	2.10 (2.00 to 2.25)	2.80 (2.50 to 4.25)
	Metabolite	B	95% CI	p_{FDR}^3	
1	PC ae C36:3	-0.38	(-0.58;-0.19)	0.02	ref.
2	PC ae C36:4	-0.37	(-0.58;-0.16)	0.03	ref.
3	PC aa C36:3	-0.34	(-0.54;-0.14)	0.04	ref.
4	PC aa C38:3	-0.34	(-0.53;-0.15)	0.03	ref.
5	PC ae C34:2	-0.30	(-0.50;-0.10)	0.08	ref.
6	PC ae C38:4	-0.29	(-0.49;-0.09)	0.09	ref.
7	PC aa C40:4	-0.30	(-0.51;-0.09)	0.09	ref.
8	PC ae C38:3	-0.27	(-0.46;-0.08)	0.09	ref.
9	PC ae C38:5	-0.30	(-0.50;-0.10)	0.08	ref.
10	PC ae C40:4	-0.24	(-0.44;-0.04)	0.15	ref.
11	PC aa C34:2	-0.25	(-0.47;-0.04)	0.15	ref.
12	PC aa C32:1	-0.26	(-0.47;-0.05)	0.15	ref.
13	PC aa C34:1	-0.27	(-0.48;-0.06)	0.14	ref.
14	PC ae C32:1	-0.24	(-0.45;-0.04)	0.15	ref.
15	PC ae C34:1	-0.25	(-0.45;-0.06)	0.14	ref.
				B	95% CI
				p_{FDR}^3	p_{trend}^3
				-0.37	(-0.69;-0.06) 0.39
				-0.29	(-0.63;0.04) 0.49
				-0.08	(-0.40;0.24) 0.91
				-0.11	(-0.42;0.20) 0.87
				-0.34	(-0.66;-0.01) 0.47
				-0.24	(-0.57;0.08) 0.61
				-0.26	(-0.60;0.08) 0.60
				-0.05	(-0.36;0.26) 0.92
				-0.22	(-0.55;0.11) 0.71
				-0.32	(-0.64;0.01) 0.49
				-0.12	(-0.47;0.23) 0.87
				-0.16	(-0.51;0.19) 0.82
				-0.12	(-0.46;0.23) 0.87
				-0.29	(-0.63;0.04) 0.49
				-0.19	(-0.51;0.13) 0.74
				-0.67	(-0.97;-0.36) 0.003
				-0.67	(-0.99;-0.35) 0.004
				-0.59	(-0.90;-0.28) 0.01
				-0.53	(-0.82;-0.23) 0.01
				-0.55	(-0.86;-0.24) 0.01
				-0.56	(-0.87;-0.25) 0.01
				-0.52	(-0.85;-0.20) 0.03
				-0.48	(-0.77;-0.18) 0.03
				-0.52	(-0.83;-0.21) 0.03
				-0.50	(-0.80;-0.19) 0.03
				-0.46	(-0.80;-0.13) 0.08
				-0.44	(-0.78;-0.11) 0.09
				-0.44	(-0.77;-0.10) 0.09
				-0.44	(-0.76;-0.12) 0.08
				-0.41	(-0.72;-0.11) 0.08

Diet quality index: DHD15-index^{1,4}

No. of participants Mean score (range)		195 73.8 (40.9 to 114.8)		65 58.6 (40.9 to 68.3)		65 73.5 (68.4 to 78.9)		65 89.2 (78.9 to 113.8)			
Metabolite	β	95% CI	p_{FDR}^3	β	95% CI	p_{FDR}^3	β	95% CI	p_{FDR}^3	p_{trend}^3	
1 PC aa C40:4	-0.17	(-0.28;-0.07)	0.15	ref.	-0.40	(-0.75;-0.04)	0.30	-0.50	(-0.85;-0.14)	0.29	0.37
2 PC aa C42:0	0.14	(0.04;0.24)	0.20	ref.	0.11	(-0.22;0.44)	0.85	0.43	(0.09;0.76)	0.45	0.37
3 PC aa C42:1	0.13	(0.04;0.23)	0.20	ref.	0.26	(-0.07;0.59)	0.49	0.47	(0.14;0.81)	0.29	0.37
4 PC ae C36:4	-0.14	(-0.24;-0.03)	0.20	ref.	-0.59	(-0.94;-0.24)	0.15	-0.50	(-0.85;-0.15)	0.29	0.37
5 lysoPC a C17:0	0.05	(-0.06;0.16)	0.72	ref.	0.46	(0.11;0.81)	0.21	0.35	(0.00;0.71)	0.54	0.45
6 PC aa C32:1	-0.15	(-0.25;-0.04)	0.20	ref.	-0.40	(-0.76;-0.03)	0.33	-0.39	(-0.75;-0.02)	0.54	0.45
7 PC aa C38:6	0.10	(0.00;0.21)	0.36	ref.	-0.07	(-0.42;0.27)	0.88	0.35	(0.00;0.70)	0.54	0.45
8 PC aa C40:5	-0.14	(-0.24;-0.03)	0.20	ref.	-0.29	(-0.64;0.06)	0.48	-0.38	(-0.73;-0.03)	0.54	0.45
9 PC ae C38:5	-0.11	(-0.21;-0.01)	0.29	ref.	-0.47	(-0.81;-0.13)	0.21	-0.40	(-0.74;-0.06)	0.54	0.45
10 PC ae C40:6	0.10	(0.00;0.20)	0.36	ref.	0.04	(-0.29;0.38)	0.90	0.32	(-0.02;0.66)	0.54	0.45
11 PC ae C42:3	0.09	(-0.01;0.19)	0.42	ref.	-0.02	(-0.36;0.31)	0.96	0.35	(0.02;0.69)	0.54	0.45
12 PC ae C44:6	0.10	(0.00;0.20)	0.36	ref.	0.07	(-0.27;0.41)	0.88	0.32	(-0.02;0.66)	0.54	0.45
13 Sarcosine	-0.10	(-0.21;0.00)	0.36	ref.	-0.05	(-0.41;0.31)	0.90	-0.37	(-0.73;-0.01)	0.54	0.45
14 SM (OH) C22:2	0.05	(-0.04;0.14)	0.64	ref.	0.18	(-0.13;0.49)	0.56	0.28	(-0.03;0.59)	0.56	0.45
15 C14:1	-0.12	(-0.23;-0.02)	0.26	ref.	-0.19	(-0.55;0.16)	0.61	-0.35	(-0.70;0.01)	0.54	0.45

¹Tested using multiple linear regression models analyzing associations of diet quality index (continuous and in tertiles) as independent variable and log transformed Z-standardized metabolite concentrations as dependent variable. The continuous analysis is presented per one-point and ten-point increase for the WCRF dietary score and DHD15-index, respectively. Tertile cut-off scores were 1.75 and 2.25 for the WCRF dietary score and 68.3 and 78.9 for the DHD15-index. Regression models were adjusted for sex, age, analytical batch, body mass index (continuous), smoking status, and stage; ²Adherence to the recommendations of the World Cancer Research Fund (WCRF), ranged 0 to 5; ³p-value adjusted for multiple testing, using false discovery rate (FDR); ⁴Adherence to the Dutch Healthy Diet guidelines 2015 (DHD15), ranged 0 to 130.

Several glycerophospholipids showed overlap between the investigated dietary exposures. An increasing adherence to the WCRF dietary score recommendations, as well as an increasing adherence to the DHD15-index recommendations were associated with decreasing concentrations of plasma phosphatidylcholine PC aa C32:1. Positive associations were observed between the DHD15-index and the Carnivore pattern and PC aa C38:6 concentrations. Inverse associations were observed for the WCRF dietary score, the DHD15-index and the Prudent pattern in relation to phosphatidylcholine PC aa C40:4.

Four phosphatidylcholines (PC ae C34:1, PC ae C34:2, PC ae C36:3, and PC ae C38:3) were among the top-15 metabolites associated with the WCRF dietary score and the Western pattern. Concentrations of these phosphatidylcholines were lower with increasing adherence to the WCRF dietary recommendations, while higher concentrations were observed with a higher consumption of the Western pattern. In addition, the WCRF dietary score and DHD15-index were inversely associated with two phosphatidylcholines (PC ae C36:4 and PC ae C38:5), while the opposite was observed for the Carnivore pattern. A higher DHD15-index and a higher intake of the Carnivore and Prudent pattern were associated with higher concentrations of PC ae C40:6.

Two sphingolipids overlapped between the dietary exposures. A higher consumption of the Western and Prudent pattern were both associated with a higher consumption of plasma SM (OH) C14:1. A higher DHD-15 index score reported higher concentrations of SM (OH) C22:2, and, in line, higher intakes of the Prudent pattern showed higher concentrations of SM (OH) C:22.

Sensitivity analyses

Sensitivity analyses excluding patients from whom blood was collected during or after any type of treatment, i.e. (neo-) adjuvant chemotherapy and/or surgery (n=19) and excluding stage IV patients (n=7) showed similar beta coefficients between the dietary exposures and plasma metabolite concentrations compared to the main analysis (*data not shown*).

Table 3 Top-15 plasma metabolites associated with dietary patterns, ranked by p_{trend}

		Continuous			Tertiles								
		1			2			3					
Dietary pattern: Western¹		195	65	65	65								
		No. of participants			-1.0 (-5.2 to -0.3)			0.1 (-0.2 to 0.5)			0.9 (0.5 to 2.4)		
		Mean score (range)											
	Metabolite	β	95% CI	$p_{\text{FDR}}^{2,3}$		B	95% CI	p_{FDR}^2	β	95% CI	p_{FDR}^2	P_{trend}^2	
1	PC aa C28:1	0.31	(0.18;0.44)	0.001	ref.	0.18	(-0.14;0.50)	0.97	0.59	(0.26;0.92)	0.08	0.10	
2	PC aa C36:1	0.24	(0.10;0.38)	0.01	ref.	0.24	(-0.08;0.57)	0.97	0.54	(0.20;0.89)	0.09	0.10	
3	PC ae C30:0	0.20	(0.06;0.34)	0.03	ref.	0.27	(-0.07;0.60)	0.97	0.49	(0.15;0.84)	0.09	0.10	
4	PC ae C34:0	0.24	(0.10;0.39)	0.01	ref.	0.24	(-0.09;0.58)	0.97	0.50	(0.15;0.85)	0.09	0.10	
5	PC ae C36:1	0.25	(0.12;0.39)	0.01	ref.	0.25	(-0.07;0.57)	0.97	0.51	(0.18;0.85)	0.09	0.10	
6	PC ae C38:2	0.19	(0.05;0.33)	0.04	ref.	0.32	(-0.01;0.65)	0.97	0.47	(0.13;0.81)	0.09	0.10	
7	PC ae C38:3	0.19	(0.05;0.32)	0.04	ref.	0.23	(-0.08;0.54)	0.97	0.49	(0.16;0.81)	0.09	0.10	
8	PC ae C40:2	0.23	(0.10;0.37)	0.01	ref.	0.06	(-0.27;0.38)	0.97	0.50	(0.16;0.83)	0.09	0.10	
9	SM (OH) C14:1	0.22	(0.09;0.34)	0.01	ref.	0.11	(-0.18;0.41)	0.97	0.44	(0.13;0.75)	0.09	0.10	
10	SM (OH) C24:1	0.28	(0.14;0.42)	0.01	ref.	0.06	(-0.27;0.39)	0.97	0.52	(0.18;0.87)	0.09	0.10	
11	PC ae C36:3	0.23	(0.10;0.37)	0.01	ref.	0.27	(-0.05;0.60)	0.97	0.45	(0.11;0.79)	0.09	0.10	
12	PC aa C30:0	0.23	(0.09;0.38)	0.02	ref.	0.22	(-0.13;0.57)	0.97	0.47	(0.11;0.84)	0.10	0.11	
13	PC ae C34:1	0.23	(0.10;0.37)	0.01	ref.	0.25	(-0.07;0.57)	0.97	0.44	(0.11;0.78)	0.09	0.11	
14	PC ae C34:2	0.26	(0.12;0.40)	0.01	ref.	0.25	(-0.08;0.58)	0.97	0.44	(0.09;0.78)	0.10	0.11	
15	PC ae C36:0	0.25	(0.10;0.40)	0.01	ref.	0.26	(-0.09;0.61)	0.97	0.48	(0.12;0.84)	0.09	0.11	

Dietary pattern: Carnivore¹

No. of participants		195	65	65	65							
Mean score (range)		0.0 (-5.2-1.5)	-0.9 (-5.2 to -0.1)	0.2 (-0.1 to 0.4)	0.7 (0.4 to 1.5)							
	Metabolite	β	95% CI	p_{FDR}^2	B	95% CI	p_{FDR}^2	β	95% CI	p_{FDR}^2	P_{trend}^2	
1	PC aa C38:0	0.32	(0.18;0.45)	0.001	ref.	0.31	(-0.01;-0.64)	0.91	0.67	(0.33;1.01)	0.01	0.02
2	PC ae C38:6	0.26	(0.12;0.39)	0.01	ref.	0.24	(-0.08;-0.56)	0.91	0.68	(0.35;1.01)	0.01	0.02
3	PC ae C36:5	0.18	(0.04;0.32)	0.12	ref.	0.19	(-0.14;-0.51)	0.91	0.66	(0.32;0.99)	0.01	0.05
4	PC aa C36:0	0.22	(0.09;0.36)	0.03	ref.	0.18	(-0.15;-0.52)	0.91	0.59	(0.25;0.94)	0.02	0.08
5	PC ae C38:5	0.20	(0.06;0.34)	0.05	ref.	0.24	(-0.09;-0.57)	0.91	0.58	(0.24;0.92)	0.02	0.08
6	PC aa C38:6	0.27	(0.13;0.40)	0.01	ref.	0.19	(-0.15;-0.53)	0.91	0.51	(0.16;0.86)	0.06	0.10
7	PC aa C40:6	0.29	(0.16;0.43)	0.00	ref.	0.10	(-0.23;-0.43)	0.91	0.50	(0.16;0.84)	0.06	0.10
8	PC aa C42:2	0.13	(-0.01;0.27)	0.35	ref.	0.22	(-0.11;-0.56)	0.91	0.50	(0.15;0.84)	0.06	0.10
9	PC ae C36:4	0.24	(0.10;0.38)	0.02	ref.	0.25	(-0.10;-0.59)	0.91	0.52	(0.16;0.87)	0.06	0.10
10	PC ae C40:6	0.26	(0.12;0.39)	0.01	ref.	0.19	(-0.13;-0.51)	0.91	0.51	(0.18;0.85)	0.06	0.10
11	Isoleucine	0.12	(-0.02;0.26)	0.35	ref.	0.23	(-0.10;-0.57)	0.91	0.45	(0.10;0.80)	0.10	0.10
12	Methionine	0.13	(-0.01;0.28)	0.33	ref.	0.23	(-0.11;-0.57)	0.91	0.44	(0.09;0.79)	0.10	0.10
13	Threonine	0.18	(0.04;0.33)	0.12	ref.	0.34	(-0.01;-0.68)	0.91	0.45	(0.09;0.80)	0.10	0.10
14	Tryptophan	0.18	(0.04;0.32)	0.12	ref.	0.35	(0.01;0.70)	0.91	0.49	(0.13;0.85)	0.08	0.10
15	Tyrosine	0.10	(-0.05;0.24)	0.56	ref.	0.30	(-0.05;0.65)	0.91	0.45	(0.09;0.81)	0.10	0.10

Dietary pattern: Prudent¹

No. of participants Mean score (range)		195 0.0 (-3.1;-1.8)		65 -1.1 (-3.1 to -0.3)		65 0.2 (-0.2 to 0.5)		65 1.0 (0.5 to 1.8)					
Metabolite		β	95% CI	p_{FDR}^2		β	95% CI	p_{FDR}^2		β	95% CI	p_{FDR}^2	P_{trend}^2
1	PC aa C40:4	-0.22	(-0.39;-0.06)	0.11	ref.	-0.39	(-0.74;-0.04)	0.68		-0.54	(-0.9;-0.18)	0.09	0.10
2	PC ae C30:2	0.20	(0.06;0.34)	0.10	ref.	0.42	(0.13;0.72)	0.68		0.50	(0.20;0.81)	0.09	0.10
3	SM (OH) C14:1	0.20	(0.06;0.35)	0.10	ref.	0.27	(-0.03;0.58)	0.73		0.50	(0.18;0.81)	0.09	0.10
4	SM (OH) C16:1	0.22	(0.08;0.37)	0.10	ref.	0.26	(-0.06;0.57)	0.73		0.51	(0.18;0.83)	0.09	0.10
5	C16	-0.27	(-0.44;-0.11)	0.06	ref.	-0.39	(-0.74;-0.05)	0.68		-0.56	(-0.92;-0.20)	0.09	0.10
6	SM (OH) C22:2	0.20	(0.06;0.34)	0.10	ref.	0.30	(0.00;0.6)	0.68		0.46	(0.15;0.77)	0.09	0.12
7	C18:2	-0.28	(-0.45;-0.11)	0.06	ref.	-0.05	(-0.41;0.31)	0.91		-0.54	(-0.91;-0.17)	0.09	0.12
8	C18:1	-0.28	(-0.45;-0.11)	0.06	ref.	-0.21	(-0.57;0.15)	0.91		-0.52	(-0.90;-0.15)	0.11	0.13
9	SM (OH) C22:1	0.20	(0.05;0.35)	0.11	ref.	0.29	(-0.03;0.61)	0.73		0.43	(0.10;0.76)	0.17	0.19
10	C18	-0.23	(-0.40;-0.07)	0.10	ref.	-0.32	(-0.67;0.03)	0.73		-0.47	(-0.84;-0.11)	0.17	0.19
11	lysoPC a C16:1	-0.28	(-0.45;-0.11)	0.06	ref.	-0.35	(-0.71;0.01)	0.68		-0.44	(-0.82;-0.06)	0.21	0.20
12	PC ae C40:6	0.13	(-0.03;0.29)	0.47	ref.	0.26	(-0.07;0.59)	0.73		0.42	(0.07;0.76)	0.19	0.20
13	Aspartate	-0.12	(-0.30;0.05)	0.52	ref.	-0.06	(-0.42;0.30)	0.91		-0.46	(-0.83;-0.08)	0.19	0.20
14	Tryptophan	0.18	(0.01;0.35)	0.25	ref.	0.13	(-0.23;0.48)	0.91		0.44	(0.08;0.81)	0.19	0.20
15	Sarcosine	-0.18	(-0.35;-0.01)	0.25	ref.	-0.34	(-0.70;0.01)	0.68		-0.41	(-0.78;-0.04)	0.26	0.20

¹Tested using multiple linear regression models analyzing associations of dietary patterns (continuous, using log transformed Z-standardized scores, and in tertiles) as independent variable and log transformed Z-standardized metabolite concentrations as dependent variable. The continuous analysis is presented per each increase in SD (equalling 1) for the Western, Carnivore, and Prudent pattern, respectively. Tertile cut-off scores were -0.3 and 0.5, -0.1 and 0.4, and -0.3 and 0.5 for the Western, Carnivore, and Prudent pattern, respectively. Regression models were adjusted for sex, age, analytical batch, body mass index (continuous), smoking status, and stage; ²p-value adjusted for multiple testing, using false discovery rate (FDR); ³In total, the Western pattern was statistically significantly associated (pFDR<0.05) with 35 plasma metabolites (see **Supplementary Table S2**), of which the top-15 metabolites were presented here.

Figure 2 Heatmap illustrating the observed top-15 metabolites (based on the smallest p -value for trend over tertiles) associated with the diet quality indices, i.e. the WCRF dietary score and DHD15-index, and the dietary patterns, i.e. the Western, Carnivore, and Prudent pattern. The color is correlated to the observed β values; a darker blue color corresponds with a more inverse association, while a darker red color represents a more positive association between the dietary exposure and the plasma metabolite. Statistically significant associations are presented by a black box around the cell.

Discussion

The aim of the current study was to investigate associations between the diet, evaluated using diet quality indices and dietary patterns, and plasma metabolite concentrations in colorectal cancer patients. The WCRF dietary score and the Carnivore pattern were observed to be statistically significantly associated with several long-chain phosphatidylcholines. In addition, when exploring the overlap in the top-15 metabolites for the respective dietary exposures, several dietary exposures were associated with identical long-chain phosphatidylcholines, which strengthens the hypothesis that diet and plasma metabolite concentrations might be associated in colorectal cancer patients.

Better adherence to the WCRF dietary and DHD guidelines, reflecting a healthier diet, was, in general, associated with lower concentrations of phosphatidylcholines in colorectal cancer patients in this study. In contrast, higher intakes of the Western pattern, which is generally regarded as an unhealthier diet [22, 43-46], showed higher concentrations of phosphatidylcholines. Similarly, a higher intake of the Carnivore pattern was positively associated with phosphatidylcholines in the current study, suggesting that a higher intake of a diet with red and processed meat, poultry, fish, and eggs is associated with higher levels of phosphatidylcholines. Interestingly, a study by Schmidt *et al.* reported that a vegan diet was characterized by lower concentrations of phosphatidylcholines and sphingolipids compared to a diet high in animal products [12]. A previous study among healthy participants also reported decreased lipid concentrations, including lysophosphatidylcholines and other glycerophospholipids, after a two-month intervention assigning healthy individuals to a Mediterranean diet, which is generally low in animal products, except for fish, compared to a control diet. The control diet was based on the American Heart Association guidelines [47], which recommend to consume low-fat dairy products, fish, poultry, and lean meats regularly. In line with our results and the study of Schmidt *et al.* [12], this may suggest that a higher intake of animal products is associated with higher phosphatidylcholine concentrations, also among those with colorectal cancer. Further studies are needed to elucidate whether phosphatidylcholine metabolism may play a role in the colorectal cancer continuum.

Diet quality indices and dietary patterns have been linked to colorectal cancer survival previously [22, 48, 49]. Identifying metabolites reflecting the interface between nutrition and colorectal cancer survival could potentially help in making nutritional recommendations for colorectal cancer survivors, based on solid scientific evidence. A

previous study suggested that cancer cells display an elevated production of phosphatidylcholines, as part of enhanced lipogenesis in cancer cells [50], to further promote proliferation and evade apoptosis [51]. Given our findings that diet seems to be associated with phosphatidylcholines in colorectal cancer patients, and thus may theoretically support the hypothesized neoplastic growth, further studies studying phosphatidylcholines in relation to colorectal cancer recurrence and survival might be of interest.

The main strength of the current study is that this is, to the best of our knowledge, the first study investigating the associations between dietary exposures and plasma metabolites in a diseased population, i.e. colorectal cancer patients, using different approaches. When exploring the top-15 metabolites associated with the investigated dietary exposures, several phosphatidylcholines were observed to overlap between our exposures. This may indicate that the reported associations in our study population between dietary exposures and plasma metabolites are robust findings.

One of the limitations is that non-fasted blood samples were used for the current study and, as a result, we cannot rule out the possibility that some observed associations might be related to recent occasional dietary intake [38]. Our study was also limited to the metabolites included in the kit, while other metabolites might also be associated with the various dietary exposures. Following the presented results, a lipid-focused approach is of interest when investigating the association between diet and metabolites in colorectal cancer patients in the future. Lipid species, such as phosphatidylcholines, possess different physicochemical properties [52], and the methods used in the current and previous studies [11, 12] do not allow in-depth interpretation of the individual fatty acid compositions. Lastly, our relatively small sample size did not allow comparison of, for example, different colorectal cancer stages [40] and subtypes, while associations between diet and metabolites could potentially be related to specific tumor characteristics [53].

In summary, we reported that the WCRF dietary score and the Carnivore pattern are associated with plasma concentrations of phosphatidylcholines in colorectal cancer patients. Several phosphatidylcholines were also observed to overlap between the dietary exposures when comparing the top-15 metabolites. Our findings should be replicated in larger study populations to allow more in-depth analysis regarding colorectal cancer stages and subtypes to also explore the role of nutritional metabolites in the colorectal cancer continuum. Furthermore, future studies should investigate the association between nutritional

metabolites and colorectal cancer recurrence and survival. These analyses might provide additional information about the potential underlying mechanisms of dietary intake in colorectal cancer patients, and the potential relationship with recurrence and survival.

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Ethical approval and consent to participate The study was performed in accordance with the Declaration of Helsinki. The COLON study was approved by the Committee of Research involving Human Subjects (region Arnhem-Nijmegen), the Netherlands. All participants included in the study provided a written informed consent.

Conflicting Interests Cornelia M. Ulrich (Director of the Comprehensive Cancer Center at Huntsman Cancer Institute, Salt Lake City, U.S.) oversees research funded by several pharmaceutical companies but has not received funding directly herself that could constitute a conflict to the current work. Where authors are identified as personnel of the International Agency for Research on Cancer / World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization.

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Supplementary materials

Supplementary Table S3 Food group composition

Food group	Food items
Alcohol-free beer	All types of alcohol-free beer
Beer	All types of beer
Butter, hard margarines and cooking fats	Butter, low-fat butter, margarine in pack, solid backing/frying fat, lard
Coffee	All types of coffee
Eggs	Boiled and fried eggs
Fish	All types of fish, except for fried fish
Fruits	All types of fruits
High-fat dairy	High-fat cheese, milk, yoghurt, and quark
Legumes	Legumes and vegetables such as green beans, string beans, peas
Light beverages	All types of light beverages such as light soda
Liquor	All types of liquor
Low-fat dairy	Low-fat cheese, milk, yoghurt, and quark
Medium-fat dairy	Medium-fat cheese, milk, yoghurt, and quark
Nuts and seeds	All types of nuts and seeds
Pastry and biscuits	Croissants, pancakes, cake, biscuits, pastry
Pizza	All types of pizza
Potatoes	All types of potatoes
Poultry	All types of poultry
Processed meat	Sausages, bacon, ribs, ham, cold cuts, unknown types of meat
Red meat	Steak, organ meat, beef roll, pork tenderloin, minced meat
Refined grain products	Refined grain cereals, pasta, rice, and breads
Savory bread spreads	Fish salad, salads on biscuits, yeast extract such as Marmite, peanut butter
Savory sauces	All types of savory sauces such as mayonnaise, ketchup, sate sauce and dressings based on mayonnaise
Snacks	Chips, meatballs, snack products and fried products such as fried fish, French fries, Dutch snacks
Soft margarines, liquid cooking fats, vegetable oils and dressings	Olive oil, halvarine, liquid margarine and baking/frying fat, diet halvarine, oil/vinegar dressing
Soups	All types of soups
Soy and vegetarian products	Soy milk and all types of meat substitutes
Sugar and sugary products	Sweet spreads, candy, chocolate products, sugar in coffee and tea
Tea	All types of tea
Vegetables	All types of vegetables
Water	All types of mineral water
Whole grain products	Whole grain cereals, pasta, rice, and breads
Wine	All types of wine

Supplementary Table S2 The associations between dietary exposures and plasma metabolites¹
WCRF dietary score⁵

		Continuous			Tertile 1		Tertile 2			Tertile 3			<i>P</i> _{trend}	<i>P</i> _{WDR}					
Metabolite	Abbreviation	Mean ± SD ₂	β	95% CI ³	<i>p</i> -value	<i>p</i> _{WDR} ⁴	Mean ± SD ₂	β	95% CI ³	<i>P</i> _{trend} ⁴	Mean ± SD ₂	β	95% CI ³	<i>p</i> -value	<i>p</i> _{WDR} ⁴				
ACYLCARNITIN ES																			
Carnitine	C0	36.0 ± 7.3	0.06	(-0.16;0.27)	0.592	0.802	36.7 ± 7.5	ref.	35.2 ± 7.4	-0.14	(-0.50;0.21)	0.437	0.869	35.7 ± 7.1	-0.07	(-0.41;0.27)	0.694	0.848	0.884
Acetyl carnitine	C2	6.0 ± 1.9	-0.02	(-0.24;0.19)	0.826	0.923	6.2 ± 2.3	ref.	5.6 ± 1.6	-0.19	(-0.53;0.16)	0.298	0.756	5.9 ± 1.6	-0.07	(-0.40;0.27)	0.696	0.848	0.907
Propionyl carnitine	C3	0.5 ± 0.2	0.11	(-0.10;0.32)	0.313	0.645	0.5 ± 0.1	ref.	0.5 ± 0.2	0.11	(-0.24;0.46)	0.530	0.869	0.5 ± 0.1	0.10	(-0.23;0.43)	0.562	0.794	0.820
Butyryl carnitine	C4	0.2 ± 0.1	-0.11	(-0.32;0.11)	0.333	0.665	0.2 ± 0.1	ref.	0.2 ± 0.1	0.11	(-0.24;0.47)	0.530	0.869	0.2 ± 0.1	-0.19	(-0.53;0.14)	0.264	0.520	0.495
Valeryl carnitine	C5	0.2 ± 0.1	0.02	(-0.18;0.22)	0.815	0.918	0.2 ± 0.1	ref.	0.2 ± 0.1	0.13	(-0.20;0.46)	0.433	0.869	0.2 ± 0.1	-0.01	(-0.32;0.31)	0.954	0.990	0.942
Decenyl carnitine	C10:1	0.1 ± 0.1	-0.04	(-0.26;0.17)	0.701	0.862	0.1 ± 0.1	ref.	0.1 ± 0.1	-0.20	(-0.56;0.15)	0.258	0.741	0.1 ± 0.1	0.02	(-0.32;0.35)	0.928	0.979	0.939
Tetradecenyl carnitine	C14:1	0.05 ± 0.02	-0.17	(-0.38;0.04)	0.107	0.411	0.06 ± 0.03	ref.	0.05 ± 0.02	-0.36	(-0.70;-0.02)	0.042	0.471	0.05 ± 0.02	-0.18	(-0.50;0.15)	0.297	0.560	0.631
Tetradecenyl carnitine	C14:2	0.02 ± 0.01	-0.15	(-0.36;0.06)	0.161	0.473	0.03 ± 0.01	ref.	0.02 ± 0.01	-0.31	(-0.65;0.04)	0.081	0.488	0.02 ± 0.01	-0.13	(-0.46;0.20)	0.428	0.727	0.789
Hexadecanoyl carnitine	C16	0.10 ± 0.03	-0.23	(-0.44;-0.02)	0.033	0.222	0.11 ± 0.03	ref.	0.10 ± 0.03	-0.38	(-0.72;-0.03)	0.033	0.471	0.10 ± 0.03	-0.36	(-0.69;-0.03)	0.032	0.151	0.187
Octadecanoyl carnitine	C18	0.05 ± 0.01	-0.18	(-0.40;0.03)	0.088	0.358	0.05 ± 0.01	ref.	0.05 ± 0.01	-0.36	(-0.71;-0.02)	0.041	0.471	0.05 ± 0.01	-0.29	(-0.62;0.04)	0.086	0.253	0.301
Octadecenyl carnitine	C18:1	0.09 ± 0.03	-0.12	(-0.34;0.10)	0.292	0.631	0.09 ± 0.03	ref.	0.08 ± 0.03	-0.30	(-0.66;0.06)	0.102	0.526	0.09 ± 0.03	-0.10	(-0.44;0.24)	0.573	0.794	0.836
Octadecadienyl carnitine	C18:2	0.03 ± 0.01	-0.03	(-0.25;0.19)	0.779	0.900	0.04 ± 0.01	ref.	0.03 ± 0.01	-0.21	(-0.56;0.15)	0.264	0.741	0.03 ± 0.01	-0.01	(-0.35;0.34)	0.975	0.990	0.983
AMINO ACIDS																			
Alanine	Ala	382.8 ± 100.7	0.16	(-0.05;0.38)	0.139	0.463	373.3 ± 103	ref.	400.1 ± 103.1	0.32	(-0.03;0.68)	0.077	0.488	379.0 ± 94.9	0.10	(-0.24;0.44)	0.550	0.794	0.836
Arginine	Arg	81.3 ± 23.5	0.07	(-0.15;0.28)	0.542	0.795	78.5 ± 22.6	ref.	82.6 ± 22.9	0.16	(-0.19;0.51)	0.377	0.815	83.8 ± 25.0	0.21	(-0.13;0.55)	0.221	0.479	0.495
Asparagine	Asn	43.5 ± 9.0	0.20	(-0.01;0.41)	0.061	0.273	42.2 ± 8.6	ref.	45.0 ± 9.6	0.31	(-0.03;0.66)	0.073	0.488	43.6 ± 9.0	0.22	(-0.10;0.55)	0.181	0.419	0.495
Aspartate	Asp	11.8 ± 4.0	-0.16	(-0.38;0.05)	0.142	0.463	11.9 ± 3.5	ref.	11.8 ± 4.0	-0.08	(-0.43;0.28)	0.676	0.917	11.7 ± 4.6	-0.21	(-0.55;0.13)	0.222	0.479	0.497

Citrulline	Cit	36.5 ± 10.2	-0.11 (-0.33;0.11)	0.333	0.665	36.8 ± 9.1 ref.	36.3 ± 10.6 ref.	-0.13 (-0.49;0.23)	0.487	0.869	36.2 ± 11.2	-0.08 (-0.42;0.26)	0.641	0.818	0.689	0.871
Glutamine	Gln	696.5 ± 98.5	0.07 (-0.13;0.28)	0.482	0.778	687.5 ± 102.9 ref.	710.0 ± 100.2 ref.	0.34 (0.00;0.67)	0.049	0.488	695.6 ± 91.3	0.14 (-0.18;0.46)	0.377	0.674	0.435	0.756
Glutamate	Glu	80.0 ± 29.7	-0.04 (-0.25;0.17)	0.713	0.862	80.5 ± 29.8 ref.	77.1 ± 26.9 ref.	-0.12 (-0.47;0.23)	0.505	0.869	82.0 ± 32.1	-0.04 (-0.37;0.30)	0.831	0.933	0.889	0.942
Glycine	Gly	215.6 ± 59.8	-0.02 (-0.21;0.18)	0.868	0.938	221.1 ± 70.8 ref.	211.3 ± 54.3 ref.	-0.09 (-0.41;0.24)	0.597	0.915	212.7 ± 49.0	-0.12 (-0.43;0.19)	0.463	0.756	0.482	0.789
Histidine	His	71.1 ± 12.4	0.08 (-0.13;0.29)	0.467	0.773	69.9 ± 11.1 ref.	74.5 ± 13 ref.	0.40 (0.06;0.74)	0.023	0.392	69.6 ± 13.0	0.00 (-0.33;0.32)	0.985	0.993	0.856	0.935
Isoleucine	Ile	75.4 ± 23.9	0.21 (0.01;0.42)	0.044	0.263	69.0 ± 19.5 ref.	81.0 ± 23.8 ref.	0.54 (0.21;0.87)	0.002	0.167	78.3 ± 27.4	0.42 (0.1;0.73)	0.010	0.087	0.020	0.138
Leucine	Leu	133.8 ± 42.3	0.15 (-0.06;0.36)	0.168	0.473	123.9 ± 34.9 ref.	143.7 ± 41.3 ref.	0.50 (0.17;0.84)	0.004	0.167	137.1 ± 49.1	0.30 (-0.03;0.62)	0.073	0.229	0.112	0.306
Lysine	Lys	193.5 ± 41.8	0.13 (-0.08;0.34)	0.234	0.550	186.0 ± 39.1 ref.	201.7 ± 37.9 ref.	0.43 (0.09;0.77)	0.015	0.327	195.4 ± 47.0	0.22 (-0.10;0.54)	0.186	0.421	0.247	0.518
Methionine	Met	20.20 ± 5.8	0.19 (-0.01;0.40)	0.069	0.298	19.1 ± 5.6 ref.	21.4 ± 5.5 ref.	0.45 (0.11;0.79)	0.010	0.264	20.7 ± 6.1	0.35 (0.02;0.67)	0.037	0.159	0.053	0.201
Ornithine	Orn	71.3 ± 24.6	0.03 (-0.19;0.24)	0.796	0.912	71.3 ± 26.9 ref.	71.0 ± 22.3 ref.	0.06 (-0.30;0.41)	0.759	0.917	71.6 ± 23.9	0.07 (-0.27;0.40)	0.695	0.848	0.734	0.887
Phenylalanine	Phe	62.8 ± 13.6	0.06 (-0.14;0.27)	0.548	0.795	61.1 ± 13.1 ref.	64.4 ± 12.7 ref.	0.21 (-0.13;0.55)	0.223	0.741	63.4 ± 15.0	0.15 (-0.17;0.48)	0.362	0.655	0.418	0.737
Proline	Pro	242.6 ± 79.5	0.21 (0.00;0.42)	0.051	0.263	224.2 ± 71.8 ref.	266.6 ± 84.9 ref.	0.52 (0.19;0.86)	0.003	0.167	243.8 ± 79.0	0.30 (-0.02;0.63)	0.065	0.217	0.100	0.301
Serine	Ser	93 ± 20.3	0.00 (-0.22;0.21)	0.994	0.994	92.8 ± 19.9 ref.	93.7 ± 20.8 ref.	-0.01 (-0.36;0.35)	0.964	0.965	92.6 ± 20.8	-0.05 (-0.39;0.29)	0.781	0.902	0.750	0.897
Threonine	Thr	108.7 ± 27.7	0.15 (-0.06;0.36)	0.165	0.473	105.3 ± 28.5 ref.	112.9 ± 27.0 ref.	0.33 (-0.01;0.68)	0.060	0.488	109.1 ± 27.3	0.24 (-0.08;0.57)	0.147	0.362	0.183	0.443
Tryptophan	Trp	53.1 ± 11.0	0.08 (-0.13;0.30)	0.442	0.759	52.2 ± 10.0 ref.	54.6 ± 10.6 ref.	0.18 (-0.17;0.53)	0.316	0.756	52.9 ± 12.4	0.01 (-0.33;0.34)	0.968	0.990	0.983	0.983
Tyrosine	Tyr	74.8 ± 24.1	0.06 (-0.15;0.28)	0.569	0.802	71.5 ± 21.4 ref.	78.2 ± 24.1 ref.	0.27 (-0.08;0.62)	0.132	0.591	75.7 ± 27.0	0.11 (-0.23;0.44)	0.527	0.794	0.592	0.820
Valine	Val	242.1 ± 64.0	0.09 (-0.11;0.30)	0.380	0.707	229.4 ± 53.6 ref.	258.4 ± 70.6 ref.	0.46 (0.12;0.79)	0.009	0.264	242.9 ± 67.2	0.20 (-0.13;0.52)	0.234	0.489	0.309	0.592
 BIOGENIC AMINES																
Creatinine	Creatinine	74.5 ± 17.4	0.04 (-0.14;0.21)	0.672	0.842	74.6 ± 15.5 ref.	73.4 ± 14.6 ref.	0.01 (-0.27;0.30)	0.927	0.955	75.3 ± 21.5	0.08 (-0.19;0.36)	0.551	0.794	0.531	0.816
Kynurenine	Kynurenine	2.7 ± 0.7	-0.12 (-0.32;0.08)	0.244	0.563	2.7 ± 0.8 ref.	2.6 ± 0.7 ref.	-0.09 (-0.43;0.24)	0.582	0.910	2.7 ± 0.8	-0.09 (-0.41;0.23)	0.600	0.796	0.665	0.856
Sarcosine	Sarcosine	4.4 ± 1.1	-0.21 (-0.42;0.01)	0.061	0.273	4.6 ± 1.2 ref.	4.3 ± 0.9 ref.	-0.26 (-0.61;0.09)	0.149	0.606	4.3 ± 1.1	-0.28 (-0.61;0.06)	0.105	0.292	0.108	0.306
Serotonin	Serotonin	0.8 ± 0.6	-0.08 (-0.29;0.14)	0.476	0.778	0.9 ± 0.6 ref.	0.7 ± 0.4 ref.	-0.23 (-0.59;0.12)	0.193	0.708	0.7 ± 0.6	-0.12 (-0.46;0.22)	0.482	0.770	0.529	0.816

Taurine	120.9 ± 49.4	-0.03	0.815	0.918	125.4 ± 51.9	ref.	114.3 ± 47	-0.24	(-0.59;0.12)	0.196	0.708	121.1 ± 48.4	-0.09	(-0.43;0.25)	0.588	0.794	0.660	0.856
Taurine																		
Trans 4-Hydroxyproline	10.2 ± 5.0	-0.08	(-0.29;0.13)	0.450	0.759	10.4 ± 4.4	ref.	10.1 ± 4.5	0.04	(-0.31;0.38)	0.834	9.939	-0.14	(-0.47;0.19)	0.402	0.691	0.348	0.631
Asymmetric dimethylarginine	0.5 ± 0.1	0.06	(-0.14;0.25)	0.578	0.802	0.5 ± 0.1	ref.	0.4 ± 0.1	-0.03	(-0.35;0.29)	0.851	0.942	0.14	(-0.16;0.45)	0.362	0.655	0.336	0.625
Symmetric dimethylarginine	0.5 ± 0.1	0.02	(-0.18;0.22)	0.863	0.938	0.5 ± 0.1	ref.	0.4 ± 0.1	-0.02	(-0.35;0.31)	0.908	0.955	0.09	(-0.22;0.40)	0.576	0.794	0.575	0.820
GLYCEROPHOSPHOLIPID S																		
Lysophosphatidylcholine (LPC) C16:0	90.4 ± 20.1	-0.04	(-0.26;0.18)	0.714	0.862	91.9 ± 20.7	ref.	93.0 ± 21.6	0.07	(-0.29;0.43)	0.687	0.917	-0.26	(-0.60;0.09)	0.143	0.362	0.133	0.344
Lysophosphatidylcholine (LPC) C16:1	2.6 ± 0.9	-0.14	(-0.36;0.08)	0.207	0.514	2.7 ± 1.0	ref.	2.5 ± 0.8	-0.18	(-0.54;0.18)	0.324	0.761	-0.35	(-0.69;-0.01)	0.047	0.185	0.051	0.201
Lysophosphatidylcholine (LPC) C17:0	1.6 ± 0.5	0.05	(-0.16;0.26)	0.654	0.840	1.6 ± 0.5	ref.	1.6 ± 0.5	0.17	(-0.17;0.52)	0.330	0.762	-0.07	(-0.40;0.26)	0.692	0.848	0.672	0.858
Lysophosphatidylcholine (LPC) C18:0	26.4 ± 6.8	-0.05	(-0.27;0.16)	0.632	0.834	26.8 ± 7.5	ref.	27.1 ± 7.6	0.02	(-0.33;0.37)	0.916	0.955	-0.19	(-0.52;0.15)	0.277	0.538	0.274	0.551
Lysophosphatidylcholine (LPC) C18:1	17.9 ± 5.4	0.00	(-0.21;0.22)	0.991	0.994	18.4 ± 5.5	ref.	17.8 ± 5.4	-0.05	(-0.40;0.30)	0.780	0.917	-0.09	(-0.43;0.25)	0.608	0.799	0.620	0.836
Lysophosphatidylcholine (LPC) C18:2	29.2 ± 10.4	0.11	(-0.09;0.32)	0.289	0.631	29.6 ± 10.0	ref.	29.1 ± 11.1	-0.02	(-0.36;0.33)	0.927	0.955	0.04	(-0.29;0.37)	0.813	0.931	0.821	0.924
Lysophosphatidylcholine (LPC) C20:3	2.0 ± 0.7	-0.06	(-0.28;0.15)	0.557	0.795	2.1 ± 0.8	ref.	2.0 ± 0.6	-0.05	(-0.40;0.30)	0.765	0.917	-0.21	(-0.54;0.13)	0.226	0.481	0.234	0.497
Lysophosphatidylcholine (LPC) C20:4	5.9 ± 1.7	0.11	(-0.10;0.33)	0.305	0.638	5.8 ± 1.6	ref.	5.8 ± 1.5	0.05	(-0.31;0.40)	0.800	0.923	0.06	(-0.28;0.39)	0.746	0.881	0.733	0.887
Lysophosphatidylcholine (LPC) C24:0	0.14 ± 0.03	-0.01	(-0.21;0.18)	0.888	0.944	0.14 ± 0.04	ref.	0.14 ± 0.03	0.03	(-0.29;0.35)	0.846	0.942	-0.05	(-0.36;0.26)	0.755	0.881	0.778	0.907
Lysophosphatidylcholine (LPC) C28:1	0.3 ± 0.1	-0.05	(-0.23;0.12)	0.545	0.795	0.3 ± 0.1	ref.	0.3 ± 0.1	0.07	(-0.22;0.36)	0.637	0.915	-0.12	(-0.39;0.16)	0.398	0.691	0.395	0.706
PC (diacyl) C28:1	3.0 ± 0.8	-0.16	(-0.36;0.04)	0.117	0.436	3.0 ± 0.7	ref.	3.1 ± 0.8	0.04	(-0.28;0.37)	0.795	0.923	-0.29	(-0.60;0.01)	0.063	0.217	0.064	0.225
PC (diacyl) C28:1	4.0 ± 1.3	-0.15	(-0.37;0.06)	0.162	0.473	4.0 ± 1.2	ref.	4.3 ± 1.5	0.14	(-0.21;0.49)	0.431	0.869	-0.26	(-0.60;0.07)	0.126	0.343	0.109	0.306
PC (diacyl) C30:0	26.6 ± 5.5	-0.14	(-0.35;0.07)	0.183	0.480	26.9 ± 4.5	ref.	26.9 ± 5.7	-0.02	(-0.36;0.32)	0.918	0.955	-0.25	(-0.57;0.08)	0.135	0.354	0.122	0.327
PC (diacyl) C32:0	32.0 ± 16.2	-0.26	(-0.47;-0.05)	0.019	0.153	34.3 ± 17.3	ref.	32.2 ± 16.2	-0.16	(-0.51;0.19)	0.377	0.815	-0.44	(-0.78;-0.11)	0.010	0.087	0.010	0.089
PC (diacyl) C32:1	8.3 ± 3.1	-0.15	(-0.36;0.07)	0.181	0.480	8.4 ± 2.7	ref.	8.8 ± 3.5	0.02	(-0.32;0.37)	0.890	0.955	-0.30	(-0.64;0.03)	0.073	0.229	0.076	0.248
PC (diacyl) C32:2	0.9 ± 0.2	-0.01	(-0.20;0.19)	0.950	0.974	0.9 ± 0.2	ref.	0.9 ± 0.3	0.07	(-0.25;0.39)	0.682	0.917	-0.12	(-0.43;0.19)	0.455	0.753	0.483	0.789
PC (diacyl) C32:3																		

PC (diacyl) C34:1	PC aa C34:1	365.4 ± 82.8	-0.27 (-0.48;-0.06)	0.013	0.145	376.5 ± 78.8	ref.	367.1 ± 84.2	-0.12 (-0.46;0.23)	0.515	0.869	350.1 ± 85.4	-0.44 (-0.77;-0.10)	0.011	0.087	0.010	0.089
PC (diacyl) C34:2	PC aa C34:2	739.8 ± 128.1	-0.25 (-0.47;-0.04)	0.022	0.155	759.8 ± 116.0	ref.	749.6 ± 129.0	-0.12 (-0.47;0.23)	0.511	0.869	706.0 ± 136.6	-0.46 (-0.80;-0.13)	0.007	0.081	0.006	0.075
PC (diacyl) C34:3	PC aa C34:3	30.1 ± 10.2	-0.21 (-0.42;0.00)	0.048	0.263	31.2 ± 10.0	ref.	30.9 ± 10.8	-0.10 (-0.44;0.25)	0.584	0.910	28.1 ± 9.6	-0.40 (-0.72;-0.07)	0.018	0.128	0.016	0.129
PC (diacyl) C34:4	PC aa C34:4	3.1 ± 1.2	-0.11 (-0.32;0.10)	0.302	0.638	3.1 ± 1.1	ref.	3.3 ± 1.3	0.13 (-0.21;-0.48)	0.446	0.869	2.9 ± 1.1	-0.28 (-0.61;-0.05)	0.095	0.271	0.090	0.282
PC (diacyl) C36:0	PC aa C36:0	3.2 ± 1.0	-0.01 (-0.22;0.20)	0.916	0.967	3.2 ± 0.9	ref.	3.3 ± 1.0	0.16 (-0.18;0.50)	0.354	0.805	3.2 ± 1.2	-0.05 (-0.38;0.27)	0.755	0.881	0.719	0.884
PC (diacyl) C36:1	PC aa C36:1	62.9 ± 17.8	-0.24 (-0.44;-0.04)	0.019	0.153	64.6 ± 18.1	ref.	63.8 ± 18.4	-0.06 (-0.39;0.28)	0.736	0.917	60.1 ± 16.7	-0.34 (-0.66;-0.02)	0.036	0.159	0.034	0.172
PC (diacyl) C36:2	PC aa C36:2	384.7 ± 79.7	-0.24 (-0.44;-0.04)	0.020	0.153	393.7 ± 77.0	ref.	388.4 ± 80.8	-0.11 (-0.44;0.23)	0.532	0.869	370.3 ± 81.3	-0.36 (-0.67;-0.04)	0.029	0.151	0.027	0.153
PC (diacyl) C36:3	PC aa C36:3	192.6 ± 43.8	-0.34 (-0.54;-0.14)	0.001	0.036	201 ± 41.4	ref.	197.4 ± 45.4	-0.08 (-0.40;0.24)	0.629	0.915	177.8 ± 42.2	-0.59 (-0.90;-0.28)	0.0003	0.011	0.0002	0.010
PC (diacyl) C36:4	PC aa C36:4	291.5 ± 72.0	-0.11 (-0.32;0.09)	0.277	0.619	291.8 ± 65.6	ref.	301.1 ± 79.5	0.08 (-0.25;0.42)	0.635	0.915	282.3 ± 72.6	-0.30 (-0.62;0.02)	0.072	0.229	0.068	0.226
PC (diacyl) C36:5	PC aa C36:5	45.8 ± 27.8	0.06 (-0.15;0.28)	0.558	0.795	43.8 ± 25.3	ref.	47.1 ± 21.9	0.32 (-0.03;0.67)	0.075	0.488	47.1 ± 34.9	0.07 (-0.27;0.40)	0.702	0.848	0.786	0.908
PC (diacyl) C36:6	PC aa C36:6	1.5 ± 0.6	0.05 (-0.16;0.26)	0.658	0.840	1.5 ± 0.6	ref.	1.6 ± 0.6	0.30 (-0.04;0.64)	0.089	0.488	1.5 ± 0.7	-0.02 (-0.35;0.31)	0.908	0.979	0.851	0.935
PC (diacyl) C38:0	PC aa C38:0	2.9 ± 0.9	-0.06 (-0.27;0.14)	0.555	0.795	2.8 ± 0.8	ref.	2.9 ± 0.8	0.06 (-0.27;0.40)	0.714	0.917	2.9 ± 1.0	-0.09 (-0.41;0.23)	0.576	0.794	0.574	0.820
PC (diacyl) C38:3	PC aa C38:3	53.2 ± 13.6	-0.34 (-0.53;-0.15)	0.001	0.026	55.1 ± 13.9	ref.	54.4 ± 14.2	-0.11 (-0.42;0.20)	0.487	0.869	49.7 ± 12.2	-0.53 (-0.82;-0.23)	0.001	0.013	0.001	0.020
PC (diacyl) C38:4	PC aa C38:4	131.5 ± 35.0	-0.12 (-0.32;0.07)	0.218	0.530	131 ± 32.5	ref.	133.4 ± 36.5	-0.02 (-0.34;0.30)	0.909	0.955	130.4 ± 37.0	-0.23 (-0.54;0.07)	0.132	0.353	0.136	0.344
PC (diacyl) C38:5	PC aa C38:5	66.2 ± 19.0	-0.05 (-0.25;0.16)	0.656	0.840	65.9 ± 17.9	ref.	68.2 ± 18.6	0.19 (-0.14;0.52)	0.264	0.741	64.9 ± 20.9	-0.16 (-0.48;0.15)	0.310	0.577	0.276	0.551
PC (diacyl) C38:6	PC aa C38:6	101.1 ± 33.6	0.08 (-0.12;0.29)	0.430	0.759	95 ± 27.6	ref.	107.1 ± 36.7	0.33 (-0.01;0.67)	0.059	0.488	103.3 ± 36.7	0.08 (-0.24;0.41)	0.625	0.813	0.698	0.874
PC (diacyl) C40:2	PC aa C40:2	0.3 ± 0.1	-0.04 (-0.24;0.17)	0.729	0.867	0.3 ± 0.1	ref.	0.3 ± 0.1	0.10 (-0.24;0.43)	0.571	0.910	0.3 ± 0.1	-0.10 (-0.42;0.22)	0.541	0.794	0.496	0.800
PC (diacyl) C40:3	PC aa C40:3	0.4 ± 0.1	-0.07 (-0.27;0.13)	0.493	0.786	0.4 ± 0.1	ref.	0.4 ± 0.1	0.04 (-0.29;0.37)	0.806	0.923	0.4 ± 0.2	-0.07 (-0.38;0.25)	0.671	0.848	0.628	0.836
PC (diacyl) C40:4	PC aa C40:4	3.0 ± 0.8	-0.30 (-0.51;-0.09)	0.005	0.085	3.2 ± 0.9	ref.	3.0 ± 0.8	-0.26 (-0.60;0.08)	0.139	0.602	2.8 ± 0.7	-0.52 (-0.85;-0.20)	0.002	0.026	0.002	0.036
PC (diacyl) C40:5	PC aa C40:5	8.3 ± 2.4	-0.20 (-0.41;0.01)	0.058	0.273	8.5 ± 2.6	ref.	8.5 ± 2.6	-0.02 (-0.36;0.32)	0.909	0.955	7.8 ± 2.0	-0.37 (-0.69;-0.04)	0.028	0.151	0.025	0.151
PC (diacyl) C40:6	PC aa C40:6	28.3 ± 10.2	0.06 (-0.14;0.27)	0.552	0.795	26.4 ± 8.6	ref.	29.6 ± 10.8	0.27 (-0.07;0.61)	0.117	0.560	29.6 ± 11.2	0.12 (-0.21;0.44)	0.482	0.770	0.536	0.816
PC (diacyl) C42:0	PC aa C42:0	0.3 ± 0.1	0.10 (-0.10;0.30)	0.349	0.667	0.3 ± 0.1	ref.	0.4 ± 0.1	0.17 (-0.16;0.50)	0.305	0.756	0.4 ± 0.1	0.12 (-0.19;0.43)	0.452	0.753	0.454	0.779

PC (diacyl) C42:1	PC aa C42:1	0.18 ± 0.05	0.02	-0.18;0.22)	0.863	0.938	0.17 ± 0.05	ref.	0.18 ± 0.05	0.06	(-0.27;-0.39)	0.716	0.917	0.2 ± 0.1	0.00	(-0.31;-0.31)	0.993	0.954	0.978
PC (diacyl) C42:2	PC aa C42:2	0.16 ± 0.04	0.08	(-0.13;0.29)	0.450	0.759	0.15 ± 0.04	ref.	0.16 ± 0.04	0.10	(-0.24;0.44)	0.571	0.910	0.16 ± 0.05	0.10	(-0.22;0.43)	0.533	0.507	0.809
PC (diacyl) C42:4	PC aa C42:4	0.10 ± 0.02	-0.12	(-0.34;0.09)	0.265	0.601	0.11 ± 0.02	ref.	0.10 ± 0.02	-0.11	(-0.47;0.24)	0.531	0.869	0.10 ± 0.02	-0.30	(-0.63;0.04)	0.087	0.253	0.282
PC (diacyl) C42:5	PC aa C42:5	0.3 ± 0.1	-0.05	(-0.26;0.17)	0.678	0.842	0.3 ± 0.1	ref.	0.3 ± 0.1	0.15	(-0.20;0.50)	0.406	0.864	0.3 ± 0.1	-0.08	(-0.42;0.26)	0.640	0.589	0.820
PC (diacyl) C42:6	PC aa C42:6	0.4 ± 0.1	-0.01	(-0.22;0.21)	0.952	0.974	0.4 ± 0.1	ref.	0.4 ± 0.1	0.09	(-0.26;0.44)	0.617	0.915	0.4 ± 0.1	0.03	(-0.30;0.36)	0.851	0.848	0.935
PC (acyl-alkyl) C30:0	PC ae C30:0	0.3 ± 0.1	-0.21	(-0.41;0.00)	0.050	0.263	0.3 ± 0.1	ref.	0.4 ± 0.1	-0.01	(-0.35;0.32)	0.943	0.958	0.3 ± 0.1	-0.36	(-0.68;-0.05)	0.026	0.145	0.151
PC (acyl-alkyl) C30:2	PC ae C30:2	0.08 ± 0.02	0.01	(-0.17;0.19)	0.925	0.968	0.08 ± 0.02	ref.	0.09 ± 0.02	0.17	(-0.13;0.46)	0.272	0.741	0.08 ± 0.02	-0.08	(-0.36;0.21)	0.592	0.794	0.820
PC (acyl-alkyl) C32:1	PC ae C32:1	5.3 ± 1.2	-0.24	(-0.45;-0.04)	0.019	0.153	5.4 ± 1.1	ref.	5.2 ± 1.2	-0.29	(-0.63;0.04)	0.085	0.488	5.1 ± 1.4	-0.44	(-0.76;-0.12)	0.007	0.081	0.089
PC (acyl-alkyl) C32:2	PC ae C32:2	1.4 ± 0.4	-0.15	(-0.35;0.05)	0.141	0.463	1.4 ± 0.3	ref.	1.4 ± 0.3	-0.15	(-0.47;0.17)	0.368	0.815	1.3 ± 0.4	-0.31	(-0.62;-0.01)	0.048	0.185	0.201
PC (acyl-alkyl) C34:0	PC ae C34:0	2.5 ± 0.6	-0.14	(-0.34;0.07)	0.200	0.514	2.5 ± 0.6	ref.	2.5 ± 0.7	0.03	(-0.31;0.36)	0.878	0.955	2.4 ± 0.7	-0.25	(-0.57;0.08)	0.138	0.356	0.344
PC (acyl-alkyl) C34:1	PC ae C34:1	18.0 ± 3.9	-0.25	(-0.45;-0.06)	0.011	0.137	18.5 ± 3.5	ref.	18.2 ± 4.3	-0.19	(-0.51;0.13)	0.235	0.741	17.3 ± 3.9	-0.41	(-0.72;-0.11)	0.009	0.083	0.089
PC (acyl-alkyl) C34:2	PC ae C34:2	20.2 ± 5.3	-0.30	(-0.50;-0.10)	0.004	0.084	21.5 ± 5.3	ref.	20.0 ± 5.1	-0.34	(-0.66;-0.01)	0.042	0.471	18.8 ± 5.3	-0.55	(-0.86;-0.24)	0.001	0.013	0.021
PC (acyl-alkyl) C34:3	PC ae C34:3	13.1 ± 4.0	-0.20	(-0.40;0.00)	0.056	0.273	13.8 ± 3.8	ref.	13.2 ± 3.9	-0.21	(-0.54;0.12)	0.212	0.741	12.3 ± 4	-0.43	(-0.74;-0.11)	0.009	0.083	0.089
PC (acyl-alkyl) C36:0	PC ae C36:0	1.2 ± 0.3	-0.26	(-0.47;-0.05)	0.017	0.153	1.2 ± 0.3	ref.	1.2 ± 0.3	-0.13	(-0.48;0.22)	0.463	0.869	1.1 ± 0.3	-0.34	(-0.67;0.00)	0.048	0.185	0.201
PC (acyl-alkyl) C36:1	PC ae C36:1	11.5 ± 2.7	-0.16	(-0.36;0.03)	0.105	0.411	11.6 ± 2.7	ref.	11.9 ± 2.8	0.05	(-0.27;0.38)	0.745	0.917	11.0 ± 2.6	-0.30	(-0.61;0.01)	0.057	0.207	0.201
PC (acyl-alkyl) C36:2	PC ae C36:2	21.7 ± 5.4	-0.15	(-0.34;0.04)	0.128	0.451	22.2 ± 5.5	ref.	22.0 ± 5.2	-0.06	(-0.38;0.26)	0.707	0.917	20.9 ± 5.4	-0.30	(-0.60;0.00)	0.055	0.205	0.201
PC (acyl-alkyl) C36:3	PC ae C36:3	11.4 ± 2.8	-0.38	(-0.58;-0.19)	0.0001	0.019	12.2 ± 2.8	ref.	11.3 ± 2.8	-0.37	(-0.69;-0.06)	0.022	0.392	10.4 ± 2.7	-0.67	(-0.97;-0.36)	0.00002	0.003	0.00004
PC (acyl-alkyl) C36:4	PC ae C36:4	26.3 ± 6.4	-0.37	(-0.58;-0.16)	0.001	0.026	27.8 ± 5.9	ref.	26.4 ± 6.6	-0.29	(-0.63;0.04)	0.091	0.488	24.3 ± 6.3	-0.67	(-0.99;-0.35)	0.0001	0.004	0.006
PC (acyl-alkyl) C36:5	PC ae C36:5	18.3 ± 5.0	-0.15	(-0.35;0.06)	0.155	0.473	18.8 ± 4.5	ref.	18.4 ± 5	-0.09	(-0.42;0.25)	0.608	0.915	17.6 ± 5.4	-0.35	(-0.67;-0.03)	0.033	0.151	0.183
PC (acyl-alkyl) C38:0	PC ae C38:0	2.5 ± 0.8	0.04	(-0.16;0.25)	0.679	0.842	2.4 ± 0.7	ref.	2.6 ± 0.8	0.28	(-0.06;0.61)	0.108	0.535	2.5 ± 0.9	0.02	(-0.31;0.34)	0.926	0.979	0.983
PC (acyl-alkyl) C38:2	PC ae C38:2	2.2 ± 0.6	-0.20	(-0.40;0.00)	0.047	0.263	2.3 ± 0.5	ref.	2.2 ± 0.6	-0.13	(-0.45;0.20)	0.452	0.869	2.1 ± 0.6	-0.37	(-0.69;-0.06)	0.020	0.130	0.147
PC (acyl-alkyl) C38:3	PC ae C38:3	4.8 ± 1.1	-0.27	(-0.46;-0.08)	0.006	0.085	4.9 ± 1.1	ref.	4.9 ± 1.2	-0.05	(-0.36;0.26)	0.756	0.917	4.5 ± 1.0	-0.48	(-0.77;-0.18)	0.002	0.026	0.036

PC (acyl-alkyl) C38:4	PC ae C38:4	17.2 ± 3.6	-0.29	(-0.49;-0.09)	0.005	0.085	17.8 ± 3.3	ref.	17.4 ± 4.1	-0.24	(-0.57;0.08)	0.148	0.606	16.3 ± 3.5	-0.56	(-0.87;-0.25)	0.001	0.013	0.001	0.021
PC (acyl-alkyl) C38:5	PC ae C38:5	21.9 ± 4.7	-0.30	(-0.50;-0.10)	0.004	0.084	22.7 ± 4	ref.	22.0 ± 4.8	-0.22	(-0.55;0.11)	0.189	0.708	20.9 ± 5.1	-0.52	(-0.83;-0.21)	0.001	0.025	0.001	0.036
PC (acyl-alkyl) C38:6	PC ae C38:6	9.2 ± 2.6	-0.10	(-0.30;0.10)	0.343	0.665	9.2 ± 2.2	ref.	9.4 ± 2.6	0.05	(-0.28;0.38)	0.774	0.917	9.1 ± 3.1	-0.21	(-0.53;0.10)	0.192	0.429	0.195	0.459
PC (acyl-alkyl) C40:1	PC ae C40:1	1.1 ± 0.3	0.06	(-0.15;0.27)	0.593	0.802	1.1 ± 0.3	ref.	1.1 ± 0.3	0.19	(-0.15;0.54)	0.277	0.741	1.1 ± 0.3	-0.03	(-0.36;0.30)	0.858	0.936	0.819	0.924
PC (acyl-alkyl) C40:2	PC ae C40:2	1.6 ± 0.4	0.00	(-0.20;0.20)	0.989	0.994	1.6 ± 0.4	ref.	1.6 ± 0.4	0.08	(-0.26;0.41)	0.655	0.915	1.6 ± 0.4	-0.01	(-0.33;0.30)	0.941	0.985	0.956	0.978
PC (acyl-alkyl) C40:3	PC ae C40:3	0.8 ± 0.2	-0.20	(-0.39;-0.01)	0.042	0.263	0.9 ± 0.2	ref.	0.8 ± 0.2	-0.18	(-0.48;0.13)	0.268	0.741	0.8 ± 0.2	-0.35	(-0.64;-0.06)	0.021	0.130	0.028	0.153
PC (acyl-alkyl) C40:4	PC ae C40:4	2.2 ± 0.4	-0.24	(-0.44;-0.04)	0.021	0.153	2.3 ± 0.4	ref.	2.2 ± 0.5	-0.32	(-0.64;0.01)	0.057	0.488	2.1 ± 0.5	-0.50	(-0.80;-0.19)	0.002	0.026	0.003	0.040
PC (acyl-alkyl) C40:5	PC ae C40:5	3.4 ± 0.7	-0.16	(-0.35;0.04)	0.124	0.448	3.4 ± 0.6	ref.	3.4 ± 0.7	-0.12	(-0.44;0.21)	0.486	0.869	3.3 ± 0.7	-0.34	(-0.65;-0.03)	0.031	0.151	0.037	0.178
PC (acyl-alkyl) C40:6	PC ae C40:6	4.8 ± 1.2	0.03	(-0.17;0.23)	0.767	0.893	4.6 ± 1.2	ref.	4.9 ± 1.3	0.17	(-0.16;0.50)	0.315	0.756	4.8 ± 1.3	-0.02	(-0.33;0.30)	0.915	0.979	0.913	0.956
PC (acyl-alkyl) C42:1	PC ae C42:1	0.3 ± 0.1	-0.18	(-0.38;0.02)	0.079	0.333	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.19	(-0.52;0.14)	0.252	0.741	0.3 ± 0.1	-0.37	(-0.68;-0.06)	0.021	0.130	0.029	0.153
PC (acyl-alkyl) C42:2	PC ae C42:2	0.4 ± 0.1	-0.07	(-0.26;0.13)	0.513	0.795	0.4 ± 0.1	ref.	0.4 ± 0.1	-0.07	(-0.39;0.25)	0.666	0.917	0.4 ± 0.1	-0.16	(-0.47;0.14)	0.294	0.560	0.317	0.598
PC (acyl-alkyl) C42:3	PC ae C42:3	0.6 ± 0.1	-0.10	(-0.30;0.10)	0.339	0.665	0.6 ± 0.1	ref.	0.6 ± 0.1	-0.08	(-0.41;0.25)	0.637	0.915	0.5 ± 0.2	-0.22	(-0.54;0.09)	0.1639	0.392	0.185	0.443
PC (acyl-alkyl) C42:4	PC ae C42:4	0.7 ± 0.2	-0.15	(-0.35;0.06)	0.157	0.473	0.7 ± 0.1	ref.	0.7 ± 0.2	-0.30	(-0.63;0.03)	0.072	0.488	0.7 ± 0.2	-0.36	(-0.68;-0.05)	0.026	0.145	0.035	0.172
PC (acyl-alkyl) C42:5	PC ae C42:5	1.5 ± 0.3	-0.09	(-0.29;0.12)	0.416	0.754	1.6 ± 0.3	ref.	1.5 ± 0.3	-0.11	(-0.45;0.22)	0.507	0.869	1.5 ± 0.3	-0.19	(-0.51;0.13)	0.243	0.501	0.280	0.551
PC (acyl-alkyl) C44:3	PC ae C44:3	0.07 ± 0.02	-0.06	(-0.26;0.14)	0.541	0.795	0.07 ± 0.02	ref.	0.06 ± 0.01	-0.19	(-0.52;0.13)	0.251	0.741	0.07 ± 0.02	-0.11	(-0.42;0.21)	0.507	0.794	0.605	0.827
PC (acyl-alkyl) C44:4	PC ae C44:4	0.2 ± 0.1	-0.10	(-0.30;0.11)	0.359	0.678	0.2 ± 0.1	ref.	0.2 ± 0.1	-0.24	(-0.57;0.10)	0.169	0.666	0.2 ± 0.1	-0.28	(-0.60;0.04)	0.087	0.253	0.111	0.306
PC (acyl-alkyl) C44:5	PC ae C44:5	1.0 ± 0.3	-0.08	(-0.29;0.13)	0.447	0.759	1.0 ± 0.3	ref.	1.0 ± 0.3	-0.18	(-0.53;0.17)	0.310	0.756	1.0 ± 0.3	-0.19	(-0.52;0.14)	0.254	0.515	0.298	0.579
PC (acyl-alkyl) C44:6	PC ae C44:6	0.8 ± 0.2	0.06	(-0.14;0.26)	0.580	0.802	0.8 ± 0.2	ref.	0.8 ± 0.2	-0.05	(-0.39;0.28)	0.751	0.917	0.8 ± 0.2	-0.03	(-0.35;0.29)	0.859	0.936	0.923	0.959
SPHINGOLIPIDS																				
Hydroxy sphingomyelin C14:1	SM OH C14:1	4.0 ± 1.0	-0.01	(-0.19;0.18)	0.933	0.969	4.0 ± 1.0	ref.	4.1 ± 1.1	0.01	(-0.29;0.31)	0.940	0.958	4.0 ± 1.0	-0.09	(-0.37;0.20)	0.560	0.794	0.587	0.820
Hydroxy sphingomyelin C16:1	SM OH C16:1	1.9 ± 0.5	0.05	(-0.14;0.24)	0.635	0.834	1.8 ± 0.5	ref.	1.9 ± 0.5	0.07	(-0.24;0.39)	0.654	0.915	1.9 ± 0.5	0.03	(-0.27;0.33)	0.829	0.933	0.806	0.923

Hydroxy sphingomyelin C22:1	SM OH C22:1	4.4 ± 1.1	-0.08	(-0.27;0.11)	0.412	0.754	4.4 ± 1.2	ref.	4.6 ± 1.0	0.12	(-0.20;0.44)	0.458	0.869	4.3 ± 1.0	-0.17	(-0.48;0.13)	0.259	0.518	0.271	0.551
Hydroxy sphingomyelin C22:2	SM OH C22:2	3.3 ± 0.9	-0.01	(-0.20;0.17)	0.880	0.944	3.2 ± 0.9	ref.	3.5 ± 0.9	0.18	(-0.12;0.48)	0.232	0.741	3.2 ± 0.8	-0.09	(-0.38;0.19)	0.521	0.794	0.514	0.810
Hydroxy sphingomyelin C24:1	SM OH C24:1	0.3 ± 0.1	-0.13	(-0.33;0.08)	0.224	0.537	0.3 ± 0.1	ref.	0.3 ± 0.1	0.01	(-0.33;0.35)	0.965	0.965	0.3 ± 0.1	-0.24	(-0.56;0.08)	0.149	0.362	0.154	0.383
Sphingomyelin C16:0	SM C16:0	67.2 ± 13.6	-0.13	(-0.32;0.06)	0.170	0.473	68.4 ± 12.6	ref.	66.1 ± 12.4	-0.17	(-0.48;0.14)	0.292	0.756	66.5 ± 15.7	-0.21	(-0.51;0.09)	0.173	0.407	0.201	0.465
Sphingomyelin C16:1	SM C16:1	9.5 ± 2.2	-0.12	(-0.30;0.06)	0.177	0.480	9.5 ± 2.2	ref.	9.6 ± 2.3	-0.06	(-0.35;0.24)	0.706	0.917	9.2 ± 2.1	-0.30	(-0.58;-0.02)	0.038	0.160	0.047	0.201
Sphingomyelin C18:0	SM C18:0	12.0 ± 3.3	0.04	(-0.16;0.23)	0.731	0.867	11.6 ± 2.8	ref.	12.0 ± 3.2	0.06	(-0.27;0.39)	0.719	0.917	12.5 ± 3.9	0.09	(-0.23;0.40)	0.592	0.794	0.566	0.820
Sphingomyelin C18:1	SM C18:1	5.7 ± 1.6	0.02	(-0.17;0.20)	0.854	0.938	5.5 ± 1.5	ref.	5.8 ± 1.8	0.05	(-0.26;0.35)	0.769	0.917	5.8 ± 1.7	-0.03	(-0.32;0.26)	0.835	0.933	0.858	0.935
Sphingomyelin C20:2	SM C20:2	0.2 ± 0.1	0.05	(-0.14;0.24)	0.612	0.820	0.15 ± 0.05	ref.	0.1 ± 0.1	-0.19	(-0.50;0.13)	0.241	0.741	0.15 ± 0.05	0.01	(-0.30;0.31)	0.971	0.990	0.870	0.939
Sphingomyelin C24:0	SM C24:0	6.1 ± 1.4	-0.26	(-0.46;-0.06)	0.011	0.137	6.4 ± 1.4	ref.	6.2 ± 1.4	-0.08	(-0.40;0.25)	0.655	0.915	5.7 ± 1.3	-0.39	(-0.71;-0.08)	0.015	0.110	0.018	0.132
Sphingomyelin C24:1	SM C24:1	13.9 ± 3.4	-0.08	(-0.28;0.13)	0.453	0.759	14.0 ± 3.1	ref.	13.7 ± 3.0	-0.04	(-0.37;0.30)	0.820	0.931	14.0 ± 4.2	-0.05	(-0.37;0.27)	0.756	0.881	0.779	0.907
Sphingomyelin C26:0	SM C26:0	0.04 ± 0.01	-0.03	(-0.25;0.18)	0.746	0.877	0.04 ± 0.01	ref.	0.04 ± 0.01	0.12	(-0.22;0.47)	0.482	0.869	0.04 ± 0.01	0.09	(-0.24;0.42)	0.583	0.794	0.573	0.820
Sphingomyelin C26:1	SM C26:1	0.10 ± 0.04	0.14	(-0.07;0.35)	0.206	0.514	0.10 ± 0.03	ref.	0.10 ± 0.03	0.33	(-0.02;0.67)	0.067	0.488	0.1 ± 0.1	0.32	(-0.01;0.65)	0.062	0.217	0.067	0.226
HEXOSES																				
Hexoses	H1	5574.0 ± 1481.3	0.07	(-0.15;0.28)	0.538	0.795	5336.7 ± 1103.8	ref.	5805.1 ± 1707.6	0.27	(-0.08;0.62)	0.132	0.591	5659.8 ± 1644.2	0.15	(-0.19;0.48)	0.389	0.686	0.460	0.779

DHD15-index

Metabolite	Abbreviation	Mean $\pm$ SD ²	Continuous			Tertile 1		Tertile 2		Tertile 3			p_{trend}	p_{FDR}^4		
			β	95% CI ³	p -value	Mean $\pm$ SD ²	β	95% CI ³	p -value	Mean $\pm$ SD ²	β	95% CI ³			p -value	
ACYLCARNITINES																
Carnitine	C0	36.0 $\pm$ 7.3	0.04	(-0.06;0.15)	0.420	0.722	36.5 $\pm$ 8.3	ref.	34.7 $\pm$ 7.2	-0.15	(-0.51;0.21)	0.420	0.735			
Acetyl carnitine	C2	6.0 $\pm$ 1.9	-0.08	(-0.19;0.02)	0.135	0.507	6.3 $\pm$ 2.3	ref.	5.7 $\pm$ 1.8	-0.36	(-0.72;-0.01)	0.048	0.380			
Propionyl carnitine	C3	0.5 $\pm$ 0.2	0.05	(-0.06;0.15)	0.385	0.722	0.5 $\pm$ 0.2	ref.	0.4 $\pm$ 0.1	-0.22	(-0.58;0.13)	0.218	0.522			
Butyryl carnitine	C4	0.2 $\pm$ 0.1	0.04	(-0.07;0.15)	0.460	0.734	0.2 $\pm$ 0.1	ref.	0.2 $\pm$ 0.1	-0.32	(-0.68;0.04)	0.087	0.433			
Valeryl carnitine	C5	0.2 $\pm$ 0.1	0.04	(-0.06;0.14)	0.464	0.734	0.2 $\pm$ 0.1	ref.	0.2 $\pm$ 0.1	-0.07	(-0.41;0.27)	0.701	0.876			
Decenyl carnitine	C10:1	0.1 $\pm$ 0.1	0.01	(-0.10;0.12)	0.859	0.943	0.1 $\pm$ 0.1	ref.	0.12 $\pm$ 0.05	-0.10	(-0.46;0.26)	0.595	0.876			
Tetradecenyl carnitine	C14:1	0.05 $\pm$ 0.02	-0.12	(-0.23;-0.02)	0.023	0.259	0.06 $\pm$ 0.03	ref.	0.05 $\pm$ 0.03	-0.19	(-0.55;0.16)	0.286	0.607			
Tetradecadienyl carnitine	C14:2	0.02 $\pm$ 0.01	-0.10	(-0.20;0.01)	0.076	0.406	0.03 $\pm$ 0.01	ref.	0.02 $\pm$ 0.01	-0.33	(-0.68;0.03)	0.071	0.430			
Hexadecanoyl carnitine	C16	0.10 $\pm$ 0.03	-0.16	(-0.26;-0.05)	0.003	0.196	0.11 $\pm$ 0.03	ref.	0.11 $\pm$ 0.03	-0.16	(-0.52;0.19)	0.375	0.686			
Octadecanoyl carnitine	C18	0.05 $\pm$ 0.01	-0.12	(-0.22;-0.01)	0.030	0.287	0.05 $\pm$ 0.01	ref.	0.05 $\pm$ 0.01	-0.19	(-0.54;0.17)	0.312	0.624			
Octadecenyl carnitine	C18:1	0.09 $\pm$ 0.03	-0.14	(-0.25;-0.03)	0.011	0.196	0.08 $\pm$ 0.02	ref.	0.09 $\pm$ 0.04	0.08	(-0.29;0.44)	0.686	0.876			
Octadecadienyl carnitine	C18:2	0.03 $\pm$ 0.01	-0.08	(-0.19;0.03)	0.166	0.543	0.03 $\pm$ 0.01	ref.	0.04 $\pm$ 0.02	0.07	(-0.30;0.44)	0.727	0.878			
AMINO ACIDS																
Alanine	Ala	382.8 $\pm$ 100.7	0.00	(-0.11;0.11)	0.998	0.998	380.0 $\pm$ 84.8	ref.	388.1 $\pm$ 125.5	-0.02	(-0.39;0.35)	0.924	0.967			
Arginine	Arg	81.3 $\pm$ 23.5	0.10	(-0.01;0.20)	0.082	0.415	79.1 $\pm$ 20.9	ref.	79.4 $\pm$ 28.1	-0.15	(-0.51;0.21)	0.422	0.735			
Asparagine	Asn	43.5 $\pm$ 9.0	0.01	(-0.10;0.11)	0.909	0.964	43.0 $\pm$ 8.3	ref.	43.9 $\pm$ 9.4	0.01	(-0.35;0.36)	0.959	0.967			
Aspartate	Asp	11.8 $\pm$ 4.0	-0.07	(-0.18;0.04)	0.234	0.629	12.2 $\pm$ 3.9	ref.	11.9 $\pm$ 4.3	-0.12	(-0.49;0.25)	0.519	0.848			
											380.3 $\pm$ 87.9	-0.04	(-0.41;0.33)	0.840	0.935	
											85.5 $\pm$ 20.3	0.18	(-0.18;0.55)	0.323	0.702	
											43.5 $\pm$ 9.5	-0.08	(-0.44;0.27)	0.645	0.872	
											11.4 $\pm$ 3.7	-0.23	(-0.60;0.14)	0.227	0.682	
														0.238	0.677	

Citrulline	36.5 ± 10.2	0.00	(-0.11;0.11)	0.971	0.983	35.3 ± 8.1	ref.	37.9 ± 11.7	0.04	(-0.33;0.41)	0.839	0.914	36.2 ± 10.5	-0.04	(-0.41;-0.33)	0.826	0.935	0.827	0.916
Glutamine	696.5 ± 98.5	-0.01	(-0.12;0.09)	0.810	0.928	691.6 ± 108.0	ref.	699.0 ± 103.2	-0.03	(-0.38;0.32)	0.871	0.941	698.9 ± 83.9	0.00	(-0.35;-0.35)	0.991	0.991	0.933	0.979
Glutamate	80.0 ± 29.7	-0.02	(-0.13;0.08)	0.671	0.852	85.2 ± 32.9	ref.	78.8 ± 27.4	-0.07	(-0.43;0.29)	0.700	0.876	76.0 ± 28.1	-0.19	(-0.56;0.17)	0.301	0.702	0.348	0.715
Glycine	215.6 ± 59.8	-0.05	(-0.15;0.05)	0.326	0.718	210.6 ± 67.4	ref.	228.9 ± 62.3	0.25	(-0.08;0.58)	0.136	0.493	207.3 ± 46.2	-0.07	(-0.41;-0.26)	0.665	0.882	0.798	0.916
Histidine	71.1 ± 12.4	-0.02	(-0.12;0.09)	0.759	0.910	72.2 ± 11.4	ref.	68.5 ± 11.7	-0.34	(-0.69;0.02)	0.064	0.430	72.6 ± 13.8	-0.05	(-0.41;-0.30)	0.774	0.925	0.750	0.916
Isoleucine	75.4 ± 23.9	0.07	(-0.04;0.17)	0.202	0.608	74.3 ± 20.5	ref.	72.3 ± 23.9	-0.11	(-0.46;0.24)	0.540	0.857	79.5 ± 26.8	0.16	(-0.19;0.51)	0.363	0.718	0.430	0.767
Leucine	133.8 ± 42.3	0.05	(-0.05;0.16)	0.315	0.718	133.1 ± 38.2	ref.	127.3 ± 40.4	-0.09	(-0.44;0.26)	0.619	0.876	141.1 ± 47.2	0.17	(-0.18;0.53)	0.340	0.702	0.365	0.715
Lysine	193.5 ± 41.8	0.04	(-0.06;0.15)	0.425	0.722	191 ± 37.9	ref.	190.5 ± 44.1	-0.10	(-0.45;0.26)	0.597	0.876	199.0 ± 43.2	0.12	(-0.23;0.48)	0.507	0.809	0.531	0.804
Methionine	20.20 ± 5.8	0.04	(-0.06;0.15)	0.419	0.722	20.20 ± 5.2	ref.	19.4 ± 5.9	-0.18	(-0.54;0.17)	0.317	0.624	21.0 ± 6.3	0.08	(-0.28;0.43)	0.678	0.882	0.766	0.916
Ornithine	71.3 ± 24.6	-0.06	(-0.17;0.04)	0.238	0.629	70.4 ± 22.2	ref.	75.9 ± 29.7	0.07	(-0.30;0.43)	0.719	0.878	67.6 ± 20.5	-0.18	(-0.54;0.19)	0.340	0.702	0.310	0.692
Phenylalanine	62.8 ± 13.6	-0.03	(-0.13;0.07)	0.573	0.825	63.7 ± 13.4	ref.	60.5 ± 12.2	-0.34	(-0.69;0.01)	0.059	0.430	64.1 ± 15.1	-0.12	(-0.47;0.23)	0.504	0.809	0.419	0.759
Proline	242.6 ± 79.5	0.10	(0.00;0.20)	0.062	0.362	232.6 ± 77.2	ref.	237.1 ± 75.2	0.04	(-0.32;0.39)	0.832	0.914	258.1 ± 84.7	0.21	(-0.15;0.56)	0.259	0.702	0.241	0.677
Serine	93 ± 20.3	0.03	(-0.08;0.14)	0.573	0.825	92.0 ± 19.5	ref.	90.7 ± 21.3	-0.16	(-0.53;0.20)	0.379	0.686	96.4 ± 20.0	0.12	(-0.24;0.48)	0.518	0.809	0.581	0.804
Threonine	108.7 ± 27.7	0.01	(-0.10;0.12)	0.853	0.943	109.9 ± 28.7	ref.	107.3 ± 29.2	-0.11	(-0.47;0.25)	0.549	0.857	109.1 ± 25.4	-0.03	(-0.39;0.33)	0.856	0.935	0.792	0.916
Tryptophan	55.1 ± 11.0	0.04	(-0.06;0.15)	0.443	0.733	52.9 ± 11.4	ref.	51.2 ± 9.9	-0.08	(-0.44;0.28)	0.671	0.876	55.1 ± 11.4	0.20	(-0.16;0.56)	0.271	0.702	0.270	0.691
Tyrosine	74.8 ± 24.1	0.03	(-0.08;0.13)	0.627	0.852	73.8 ± 22.0	ref.	72.0 ± 22.6	-0.19	(-0.55;0.18)	0.316	0.624	78.4 ± 27.3	0.06	(-0.30;0.43)	0.733	0.909	0.781	0.916
Valine	242.1 ± 64.0	0.03	(-0.07;0.14)	0.521	0.793	243 ± 57.5	ref.	230.8 ± 60.2	-0.15	(-0.50;0.20)	0.415	0.735	252.4 ± 72.5	0.13	(-0.22;0.48)	0.468	0.775	0.495	0.794
BIOGENIC AMINES																			
Creatinine	74.5 ± 17.4	0.00	(-0.08;0.09)	0.914	0.964	75.3 ± 15.5	ref.	72.7 ± 16.8	0.04	(-0.25;0.34)	0.765	0.900	75.4 ± 19.6	0.15	(-0.15;0.44)	0.328	0.702	0.296	0.692
Kynurenine	2.7 ± 0.7	-0.02	(-0.12;0.08)	0.674	0.852	2.6 ± 0.7	ref.	2.8 ± 0.8	0.23	(-0.11;0.57)	0.192	0.520	2.7 ± 0.7	0.14	(-0.20;0.49)	0.413	0.731	0.462	0.793
Sarcosine	4.4 ± 1.1	-0.10	(-0.21;0.00)	0.062	0.362	4.6 ± 1.0	ref.	4.4 ± 1.2	-0.05	(-0.41;0.31)	0.793	0.903	4.2 ± 1.1	-0.37	(-0.73;-0.01)	0.045	0.539	0.052	0.447
Serotonin	0.8 ± 0.6	-0.05	(-0.16;0.05)	0.336	0.718	0.8 ± 0.6	ref.	0.7 ± 0.5	-0.32	(-0.68;0.04)	0.084	0.430	0.7 ± 0.6	-0.23	(-0.59;0.13)	0.216	0.674	0.181	0.623

Taurine	120.9 ± 49.4	-0.03	(-0.14;0.07)	0.542	0.815	123.7 ± 51.4	ref.	117.2 ± 50.3	-0.25	(-0.62;0.11)	0.177	0.520	121.7 ± 46.9	-0.12	(-0.48;0.25)	0.538	0.811	0.548	0.804
Trans 4-Hydroxyproline	10.2 ± 5.0	-0.04	(-0.14;0.07)	0.507	0.790	10.9 ± 4.2	ref.	9.3 ± 3.9	-0.32	(-0.67;0.03)	0.078	0.430	10.4 ± 6.4	-0.20	(-0.55;0.16)	0.282	0.702	0.259	0.681
Asymmetric dimethylarginine	0.5 ± 0.1	-0.06	(-0.16;0.04)	0.239	0.629	0.5 ± 0.1	ref.	0.5 ± 0.1	0.04	(-0.28;0.37)	0.794	0.903	0.4 ± 0.1	-0.17	(-0.50;0.16)	0.316	0.702	0.385	0.737
Symmetric dimethylarginine	0.5 ± 0.1	-0.08	(-0.18;0.02)	0.110	0.462	0.5 ± 0.1	ref.	0.5 ± 0.1	0.04	(-0.30;0.37)	0.832	0.914	0.4 ± 0.1	-0.22	(-0.56;0.12)	0.206	0.673	0.232	0.677
GLYCEROPHOSPHOLIPIDS																			
LysoPC (acyl) C16:0	90.4 ± 20.1	-0.03	(-0.14;0.08)	0.650	0.852	87.8 ± 17.8	ref.	92.6 ± 23.1	0.24	(-0.13;0.61)	0.205	0.520	90.9 ± 19.0	0.15	(-0.22;0.52)	0.435	0.747	0.348	0.715
LysoPC (acyl) C16:1	2.6 ± 0.9	-0.14	(-0.25;-0.03)	0.013	0.196	2.6 ± 1.0	ref.	2.7 ± 1.1	0.01	(-0.36;0.38)	0.970	0.970	2.4 ± 0.7	-0.16	(-0.53;0.22)	0.408	0.731	0.483	0.794
LysoPC (acyl) C17:0	1.6 ± 0.5	0.05	(-0.06;0.16)	0.352	0.722	1.4 ± 0.5	ref.	1.7 ± 0.6	0.46	(0.11;0.81)	0.011	0.207	1.6 ± 0.4	0.35	(0.00;0.71)	0.050	0.539	0.032	0.447
LysoPC (acyl) C18:0	26.4 ± 6.8	0.01	(-0.10;0.11)	0.905	0.964	25.1 ± 5.8	ref.	27.4 ± 8.6	0.22	(-0.14;0.58)	0.238	0.549	26.6 ± 5.5	0.16	(-0.20;0.52)	0.389	0.731	0.303	0.692
LysoPC (acyl) C18:1	17.9 ± 5.4	-0.05	(-0.15;0.06)	0.387	0.722	17.5 ± 5.2	ref.	18.7 ± 6.0	0.23	(-0.13;0.60)	0.206	0.520	17.7 ± 4.8	0.08	(-0.29;0.44)	0.672	0.882	0.586	0.804
LysoPC (acyl) C18:2	29.2 ± 10.4	0.03	(-0.07;0.14)	0.520	0.793	28.2 ± 10.0	ref.	29.1 ± 11.5	0.05	(-0.30;0.40)	0.781	0.903	30.5 ± 9.6	0.19	(-0.16;0.54)	0.293	0.702	0.253	0.677
LysoPC (acyl) C20:3	2.0 ± 0.7	-0.08	(-0.18;0.03)	0.155	0.531	2.0 ± 0.7	ref.	2.1 ± 0.7	0.14	(-0.22;0.50)	0.441	0.758	2.0 ± 0.6	-0.03	(-0.40;0.33)	0.857	0.935	0.985	0.985
LysoPC (acyl) C20:4	5.9 ± 1.7	-0.05	(-0.16;0.06)	0.341	0.718	5.8 ± 1.7	ref.	6.1 ± 2.0	0.23	(-0.13;0.60)	0.210	0.520	5.8 ± 1.4	0.04	(-0.32;0.41)	0.819	0.935	0.701	0.897
LysoPC (acyl) C24:0	0.14 ± 0.03	0.03	(-0.07;0.13)	0.579	0.825	0.13 ± 0.03	ref.	0.14 ± 0.04	0.22	(-0.11;0.55)	0.195	0.520	0.14 ± 0.03	0.20	(-0.13;0.53)	0.242	0.690	0.189	0.634
LysoPC (acyl) C28:1	0.3 ± 0.1	-0.02	(-0.11;0.07)	0.669	0.852	0.3 ± 0.1	ref.	0.3 ± 0.1	0.31	(0.01;0.60)	0.044	0.367	0.3 ± 0.1	0.08	(-0.21;0.38)	0.591	0.833	0.498	0.794
PC (diacyl) C28:1	3.0 ± 0.8	-0.02	(-0.12;0.08)	0.747	0.910	2.8 ± 0.7	ref.	3.1 ± 0.8	-0.02	(-0.36;0.32)	0.908	0.958	3.1 ± 0.7	-0.01	(-0.35;0.33)	0.968	0.983	0.902	0.966
PC (diacyl) C30:0	4.0 ± 1.3	-0.04	(-0.15;0.06)	0.425	0.722	4.0 ± 1.4	ref.	4.0 ± 1.3	-0.19	(-0.55;0.18)	0.312	0.624	4.1 ± 1.3	-0.10	(-0.47;0.26)	0.577	0.832	0.579	0.804
PC (diacyl) C32:0	26.6 ± 5.5	-0.03	(-0.13;0.07)	0.573	0.825	26.9 ± 6.3	ref.	25.9 ± 5.1	-0.36	(-0.71;-0.01)	0.044	0.367	27.1 ± 4.9	-0.09	(-0.44;0.26)	0.632	0.865	0.588	0.804
PC (diacyl) C32:1	32.0 ± 16.2	-0.15	(-0.25;-0.04)	0.008	0.196	35.7 ± 19.7	ref.	31.1 ± 16	-0.40	(-0.76;-0.03)	0.035	0.330	29.2 ± 11.1	-0.39	(-0.75;-0.02)	0.039	0.539	0.037	0.447
PC (diacyl) C32:2	8.3 ± 3.1	-0.04	(-0.15;0.06)	0.416	0.722	8.3 ± 3.1	ref.	8.2 ± 3.2	-0.27	(-0.63;0.09)	0.148	0.505	8.5 ± 3.0	-0.16	(-0.52;0.21)	0.402	0.731	0.503	0.794
PC (diacyl) C32:3	0.9 ± 0.2	-0.04	(-0.13;0.06)	0.466	0.734	0.9 ± 0.2	ref.	0.9 ± 0.3	-0.19	(-0.52;0.14)	0.265	0.581	0.9 ± 0.2	-0.14	(-0.47;0.19)	0.410	0.731	0.544	0.804

PC (diacyl) C34:1	PC aa C34:1	365.4 ± 82.8	-0.12	(-0.23;-0.02)	0.023	0.259	376.6 ± 89.6	ref.	361.7 ± 83.5	-0.30	(-0.66;0.06)	0.108	0.481	357.9 ± 74.8	-0.30	(-0.66;0.06)	0.106	0.578	0.103	0.555
PC (diacyl) C34:2	PC aa C34:2	739.8 ± 128.1	-0.02	(-0.13;0.08)	0.656	0.852	715.2 ± 126.7	ref.	715.9 ± 132.2	-0.48	(-0.85;-0.12)	0.010	0.207	752.2 ± 123.8	-0.19	(-0.56;0.17)	0.297	0.702	0.308	0.692
PC (diacyl) C34:3	PC aa C34:3	30.1 ± 10.2	-0.10	(-0.21;0.00)	0.061	0.362	30.6 ± 9.2	ref.	30.0 ± 12.1	-0.40	(-0.76;-0.05)	0.027	0.302	29.8 ± 9.1	-0.32	(-0.67;0.04)	0.079	0.558	0.089	0.555
PC (diacyl) C34:4	PC aa C34:4	3.1 ± 1.2	-0.09	(-0.20;0.02)	0.096	0.429	3.2 ± 1.3	ref.	3.1 ± 1.2	-0.27	(-0.63;0.08)	0.133	0.493	3.0 ± 1.1	-0.26	(-0.62;0.10)	0.159	0.607	0.218	0.664
PC (diacyl) C36:0	PC aa C36:0	3.2 ± 1.0	0.06	(-0.04;0.16)	0.248	0.637	3.2 ± 1.1	ref.	3.0 ± 0.9	-0.30	(-0.65;0.05)	0.091	0.435	3.4 ± 1.0	0.11	(-0.24;0.46)	0.528	0.809	0.498	0.794
PC (diacyl) C36:1	PC aa C36:1	62.9 ± 17.8	-0.08	(-0.18;0.03)	0.142	0.507	63.1 ± 15.9	ref.	63.1 ± 20	-0.22	(-0.56;0.13)	0.215	0.522	62.6 ± 17.4	-0.16	(-0.51;0.19)	0.364	0.718	0.368	0.715
PC (diacyl) C36:2	PC aa C36:2	384.7 ± 79.7	-0.01	(-0.11;0.09)	0.866	0.943	385.0 ± 76.2	ref.	376.8 ± 87.4	-0.39	(-0.73;-0.05)	0.027	0.302	392.4 ± 75.4	-0.14	(-0.49;0.20)	0.416	0.731	0.435	0.767
PC (diacyl) C36:3	PC aa C36:3	192.6 ± 43.8	-0.11	(-0.21;-0.01)	0.041	0.362	193.8 ± 44.1	ref.	192.7 ± 45.6	-0.28	(-0.63;0.07)	0.113	0.491	191.2 ± 42.3	-0.26	(-0.61;0.08)	0.138	0.578	0.165	0.598
PC (diacyl) C36:4	PC aa C36:4	291.5 ± 72.0	-0.10	(-0.20;0.00)	0.060	0.362	300.3 ± 79.3	ref.	287.2 ± 72.3	-0.32	(-0.67;0.03)	0.074	0.430	286.9 ± 64.0	-0.28	(-0.63;0.07)	0.113	0.578	0.145	0.598
PC (diacyl) C36:5	PC aa C36:5	45.8 ± 27.8	0.01	(-0.09;0.12)	0.787	0.910	48.5 ± 36.9	ref.	43.3 ± 20.1	-0.07	(-0.44;0.29)	0.694	0.876	45.4 ± 23.7	0.04	(-0.32;0.41)	0.827	0.935	0.833	0.916
PC (diacyl) C36:6	PC aa C36:6	1.5 ± 0.6	0.05	(-0.06;0.15)	0.392	0.722	1.5 ± 0.7	ref.	1.5 ± 0.6	-0.09	(-0.44;0.27)	0.629	0.876	1.6 ± 0.7	0.17	(-0.19;0.53)	0.347	0.705	0.280	0.691
PC (diacyl) C38:0	PC aa C38:0	2.9 ± 0.9	0.09	(-0.02;0.19)	0.101	0.437	2.7 ± 0.9	ref.	2.8 ± 0.8	-0.08	(-0.42;0.26)	0.652	0.876	3.1 ± 0.9	0.24	(-0.10;0.58)	0.174	0.624	0.171	0.603
PC (diacyl) C38:3	PC aa C38:3	53.2 ± 13.6	-0.12	(-0.21;-0.02)	0.019	0.252	53.1 ± 12.9	ref.	53.9 ± 14.1	-0.21	(-0.54;0.11)	0.202	0.520	52.6 ± 14	-0.25	(-0.58;0.08)	0.132	0.578	0.158	0.598
PC (diacyl) C38:4	PC aa C38:4	131.5 ± 35.0	-0.09	(-0.19;0.00)	0.056	0.362	133.4 ± 38.7	ref.	132.2 ± 34.1	-0.21	(-0.54;0.12)	0.209	0.520	129.1 ± 32.2	-0.25	(-0.58;0.08)	0.133	0.578	0.165	0.598
PC (diacyl) C38:5	PC aa C38:5	66.2 ± 19.0	-0.07	(-0.17;0.04)	0.208	0.608	67.6 ± 21.6	ref.	65.4 ± 18.3	-0.24	(-0.58;0.10)	0.176	0.520	65.7 ± 17.2	-0.15	(-0.50;0.19)	0.379	0.730	0.409	0.759
PC (diacyl) C38:6	PC aa C38:6	101.1 ± 33.6	0.10	(0.00;0.21)	0.056	0.362	96.0 ± 34.9	ref.	97.1 ± 29.3	-0.07	(-0.42;0.27)	0.675	0.876	110.3 ± 34.9	0.35	(0.00;0.70)	0.049	0.539	0.043	0.447
PC (diacyl) C40:2	PC aa C40:2	0.3 ± 0.1	0.05	(-0.05;0.15)	0.328	0.718	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.23	(-0.57;0.12)	0.195	0.520	0.3 ± 0.1	0.10	(-0.24;0.45)	0.561	0.817	0.596	0.807
PC (diacyl) C40:3	PC aa C40:3	0.4 ± 0.1	-0.02	(-0.12;0.08)	0.719	0.900	0.4 ± 0.2	ref.	0.4 ± 0.1	-0.22	(-0.56;0.12)	0.201	0.520	0.4 ± 0.1	-0.03	(-0.37;0.31)	0.847	0.935	0.802	0.916
PC (diacyl) C40:4	PC aa C40:4	3.0 ± 0.8	-0.17	(-0.28;-0.07)	0.001	0.153	3.2 ± 1.0	ref.	3.0 ± 0.8	-0.40	(-0.75;-0.04)	0.029	0.302	2.9 ± 0.7	-0.50	(-0.85;-0.14)	0.007	0.294	0.009	0.365
PC (diacyl) C40:5	PC aa C40:5	8.3 ± 2.4	-0.14	(-0.24;-0.03)	0.010	0.196	8.6 ± 2.7	ref.	8.3 ± 2.3	-0.29	(-0.64;0.06)	0.106	0.481	8.0 ± 2.3	-0.38	(-0.73;-0.03)	0.035	0.539	0.036	0.447
PC (diacyl) C40:6	PC aa C40:6	28.3 ± 10.2	0.08	(-0.02;0.18)	0.118	0.469	27 ± 10.9	ref.	27.4 ± 8.6	-0.05	(-0.40;0.29)	0.755	0.895	30.5 ± 10.7	0.28	(-0.06;0.63)	0.109	0.578	0.108	0.555
PC (diacyl) C42:0	PC aa C42:0	0.3 ± 0.1	0.14	(0.04;0.24)	0.006	0.196	0.3 ± 0.1	ref.	0.3 ± 0.1	0.11	(-0.22;0.44)	0.526	0.849	0.4 ± 0.1	0.43	(0.09;0.76)	0.013	0.445	0.011	0.365

PC (diacyl) C42:1	PC aa C42:1	0.18 ± 0.05	0.13	(0.04;0.23)	0.008	0.196	0.16 ± 0.05	ref.	0.18 ± 0.05	0.26	(-0.07;0.59)	0.131	0.493	0.19 ± 0.05	0.47	(0.14;0.81)	0.006	0.294	0.005	0.365
PC (diacyl) C42:2	PC aa C42:2	0.16 ± 0.04	0.04	(-0.06;0.15)	0.397	0.722	0.16 ± 0.05	ref.	0.15 ± 0.04	-0.27	(-0.61;0.08)	0.131	0.493	0.17 ± 0.04	0.18	(-0.16;0.53)	0.305	0.702	0.284	0.691
PC (diacyl) C42:4	PC aa C42:4	0.10 ± 0.02	-0.06	(-0.17;0.05)	0.295	0.718	0.11 ± 0.03	ref.	0.10 ± 0.02	-0.43	(-0.79;- 0.07)	0.022	0.302	0.11 ± 0.02	-0.12	(-0.48;0.25)	0.531	0.809	0.558	0.804
PC (diacyl) C42:5	PC aa C42:5	0.3 ± 0.1	0.00	(-0.10;0.11)	0.955	0.983	0.3 ± 0.2	ref.	0.2 ± 0.1	-0.26	(-0.62;0.10)	0.157	0.514	0.3 ± 0.1	-0.06	(-0.42;0.30)	0.749	0.910	0.698	0.897
PC (diacyl) C42:6	PC aa C42:6	0.4 ± 0.1	-0.05	(-0.15;0.06)	0.384	0.722	0.4 ± 0.2	ref.	0.4 ± 0.1	-0.27	(-0.62;0.09)	0.145	0.505	0.4 ± 0.1	-0.10	(-0.46;0.26)	0.586	0.833	0.583	0.804
PC (acyl-alkyl) C30:0	PC ae C30:0	0.3 ± 0.1	-0.02	(-0.13;0.08)	0.660	0.852	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.01	(-0.36;0.34)	0.951	0.967	0.3 ± 0.1	0.01	(-0.34;0.36)	0.948	0.977	0.883	0.961
PC (acyl-alkyl) C30:2	PC ae C30:2	0.08 ± 0.02	0.01	(-0.08;0.10)	0.759	0.910	0.08 ± 0.02	ref.	0.09 ± 0.02	0.22	(-0.08;0.53)	0.151	0.505	0.09 ± 0.02	0.12	(-0.19;0.42)	0.463	0.775	0.350	0.715
PC (acyl-alkyl) C32:1	PC ae C32:1	5.3 ± 1.2	-0.05	(-0.15;0.05)	0.343	0.718	5.2 ± 1.2	ref.	5.2 ± 1.3	-0.31	(-0.66;0.04)	0.080	0.430	5.3 ± 1.2	-0.16	(-0.50;0.19)	0.381	0.730	0.414	0.759
PC (acyl-alkyl) C32:2	PC ae C32:2	1.4 ± 0.4	-0.07	(-0.17;0.03)	0.159	0.531	1.3 ± 0.4	ref.	1.4 ± 0.4	-0.21	(-0.54;0.13)	0.226	0.530	1.4 ± 0.3	-0.17	(-0.51;0.16)	0.310	0.702	0.355	0.715
PC (acyl-alkyl) C34:0	PC ae C34:0	2.5 ± 0.6	-0.03	(-0.13;0.08)	0.636	0.852	2.4 ± 0.6	ref.	2.4 ± 0.7	-0.20	(-0.55;0.15)	0.254	0.568	2.5 ± 0.6	-0.02	(-0.37;0.33)	0.926	0.969	0.943	0.979
PC (acyl-alkyl) C34:1	PC ae C34:1	18.0 ± 3.9	-0.05	(-0.15;0.05)	0.324	0.718	17.5 ± 3.3	ref.	18.4 ± 4.7	-0.09	(-0.43;0.25)	0.599	0.876	18.2 ± 3.6	-0.06	(-0.40;0.28)	0.721	0.909	0.739	0.916
PC (acyl-alkyl) C34:2	PC ae C34:2	20.2 ± 5.3	-0.07	(-0.17;0.03)	0.189	0.603	20.4 ± 5.0	ref.	19.7 ± 5.9	-0.48	(-0.81;- 0.14)	0.006	0.207	20.6 ± 5.0	-0.27	(-0.61;0.07)	0.123	0.578	0.146	0.598
PC (acyl-alkyl) C34:3	PC ae C34:3	13.1 ± 4.0	-0.01	(-0.11;0.09)	0.851	0.943	13.0 ± 3.3	ref.	12.8 ± 4.5	-0.39	(-0.73;- 0.05)	0.025	0.302	13.6 ± 4.0	-0.14	(-0.48;0.21)	0.440	0.747	0.513	0.797
PC (acyl-alkyl) C36:0	PC ae C36:0	1.2 ± 0.3	-0.09	(-0.20;0.01)	0.089	0.424	1.2 ± 0.4	ref.	1.1 ± 0.3	-0.41	(-0.77;- 0.05)	0.027	0.302	1.1 ± 0.3	-0.29	(-0.65;0.07)	0.111	0.578	0.090	0.555
PC (acyl-alkyl) C36:1	PC ae C36:1	11.5 ± 2.7	-0.01	(-0.11;0.09)	0.786	0.910	10.9 ± 2.2	ref.	11.8 ± 3.2	0.08	(-0.26;0.42)	0.648	0.876	11.7 ± 2.5	0.08	(-0.26;0.41)	0.660	0.882	0.579	0.804
PC (acyl-alkyl) C36:2	PC ae C36:2	21.7 ± 5.4	0.04	(-0.06;0.14)	0.404	0.722	20.6 ± 4.8	ref.	22.0 ± 6.4	-0.08	(-0.41;0.25)	0.637	0.876	22.6 ± 4.7	0.11	(-0.22;0.44)	0.525	0.809	0.448	0.780
PC (acyl-alkyl) C36:3	PC ae C36:3	11.4 ± 2.8	-0.09	(-0.19;0.01)	0.075	0.406	11.5 ± 2.6	ref.	11.2 ± 3.2	-0.45	(-0.79;- 0.12)	0.008	0.207	11.4 ± 2.7	-0.32	(-0.66;0.02)	0.063	0.539	0.082	0.555
PC (acyl-alkyl) C36:4	PC ae C36:4	26.3 ± 6.4	-0.14	(-0.24;- 0.03)	0.012	0.196	27.5 ± 6.2	ref.	25.5 ± 6.4	-0.59	(-0.94;- 0.24)	0.001	0.147	25.9 ± 6.4	-0.50	(-0.85;- 0.15)	0.006	0.294	0.009	0.365
PC (acyl-alkyl) C36:5	PC ae C36:5	18.3 ± 5.0	-0.08	(-0.18;0.03)	0.144	0.507	19.1 ± 5.3	ref.	17.6 ± 4.7	-0.51	(-0.85;- 0.17)	0.004	0.207	18.3 ± 4.8	-0.32	(-0.66;0.02)	0.067	0.539	0.099	0.555
PC (acyl-alkyl) C38:0	PC ae C38:0	2.5 ± 0.8	0.06	(-0.05;0.16)	0.280	0.695	2.4 ± 0.9	ref.	2.5 ± 0.8	-0.02	(-0.37;0.32)	0.889	0.953	2.6 ± 0.8	0.25	(-0.09;0.60)	0.152	0.607	0.120	0.594
PC (acyl-alkyl) C38:2	PC ae C38:2	2.2 ± 0.6	0.00	(-0.10;0.10)	0.961	0.983	2.1 ± 0.5	ref.	2.2 ± 0.7	-0.12	(-0.46;0.23)	0.506	0.838	2.2 ± 0.5	-0.01	(-0.36;0.33)	0.933	0.969	0.970	0.985
PC (acyl-alkyl) C38:3	PC ae C38:3	4.8 ± 1.1	-0.06	(-0.16;0.04)	0.223	0.629	4.6 ± 1.0	ref.	4.9 ± 1.3	-0.01	(-0.34;0.32)	0.941	0.967	4.8 ± 1.0	-0.07	(-0.40;0.26)	0.691	0.882	0.819	0.916

PC (acyl-alkyl) C38:4	PC ae C38:4	17.2 ± 3.6	-0.09	(-0.19;0.01)	0.095	0.429	17 ± 3.3	ref.	17.4 ± 3.9	-0.23	(-0.57;0.12)	0.195	0.520	17.2 ± 3.7	-0.26	(-0.61;-0.08)	0.138	0.578	0.211	0.656
PC (acyl-alkyl) C38:5	PC ae C38:5	21.9 ± 4.7	-0.11	(-0.21;-0.01)	0.028	0.287	22.5 ± 4.7	ref.	21.6 ± 4.6	-0.47	(-0.81;-0.13)	0.007	0.207	21.8 ± 4.7	-0.40	(-0.74;-0.06)	0.022	0.539	0.034	0.447
PC (acyl-alkyl) C38:6	PC ae C38:6	9.2 ± 2.6	0.01	(-0.09;0.12)	0.775	0.910	9.2 ± 2.9	ref.	8.9 ± 2.4	-0.30	(-0.64;0.04)	0.082	0.430	9.5 ± 2.5	-0.01	(-0.35;0.33)	0.960	0.982	0.983	0.985
PC (acyl-alkyl) C40:1	PC ae C40:1	1.1 ± 0.3	0.05	(-0.06;0.15)	0.399	0.722	1.1 ± 0.3	ref.	1.1 ± 0.3	-0.10	(-0.45;0.25)	0.585	0.876	1.2 ± 0.3	0.22	(-0.14;-0.57)	0.234	0.682	0.202	0.645
PC (acyl-alkyl) C40:2	PC ae C40:2	1.6 ± 0.4	0.02	(-0.08;0.13)	0.632	0.852	1.5 ± 0.4	ref.	1.6 ± 0.4	0.07	(-0.27;0.41)	0.697	0.876	1.6 ± 0.4	0.10	(-0.24;0.45)	0.553	0.815	0.497	0.794
PC (acyl-alkyl) C40:3	PC ae C40:3	0.8 ± 0.2	-0.02	(-0.12;0.07)	0.642	0.852	0.8 ± 0.2	ref.	0.9 ± 0.2	-0.07	(-0.39;0.26)	0.689	0.876	0.9 ± 0.2	0.00	(-0.33;0.32)	0.987	0.991	0.891	0.962
PC (acyl-alkyl) C40:4	PC ae C40:4	2.2 ± 0.4	-0.04	(-0.14;0.06)	0.461	0.734	2.2 ± 0.4	ref.	2.2 ± 0.5	-0.18	(-0.52;0.16)	0.298	0.624	2.3 ± 0.5	-0.07	(-0.41;0.27)	0.691	0.882	0.784	0.916
PC (acyl-alkyl) C40:5	PC ae C40:5	3.4 ± 0.7	-0.01	(-0.11;0.09)	0.784	0.910	3.3 ± 0.6	ref.	3.4 ± 0.8	-0.18	(-0.52;0.15)	0.284	0.607	3.4 ± 0.6	-0.03	(-0.37;0.31)	0.869	0.935	0.957	0.985
PC (acyl-alkyl) C42:1	PC ae C42:1	0.3 ± 0.1	-0.07	(-0.17;0.03)	0.201	0.608	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.17	(-0.51;0.17)	0.339	0.643	0.3 ± 0.1	-0.09	(-0.43;0.26)	0.620	0.857	0.758	0.916
PC (acyl-alkyl) C42:2	PC ae C42:2	0.4 ± 0.1	0.04	(-0.06;0.14)	0.423	0.722	0.4 ± 0.1	ref.	0.4 ± 0.1	-0.10	(-0.43;0.23)	0.550	0.857	0.4 ± 0.1	0.16	(-0.17;0.49)	0.336	0.702	0.301	0.692
PC (acyl-alkyl) C42:3	PC ae C42:3	0.6 ± 0.1	0.09	(-0.01;0.19)	0.083	0.415	0.5 ± 0.1	ref.	0.5 ± 0.1	-0.02	(-0.36;0.31)	0.899	0.956	0.6 ± 0.1	0.35	(0.02;0.69)	0.0415	0.539	0.034	0.447
PC (acyl-alkyl) C42:4	PC ae C42:4	0.7 ± 0.2	0.04	(-0.06;0.15)	0.389	0.722	0.7 ± 0.1	ref.	0.7 ± 0.2	-0.04	(-0.39;0.30)	0.807	0.903	0.7 ± 0.2	0.18	(-0.17;0.52)	0.321	0.702	0.281	0.691
PC (acyl-alkyl) C42:5	PC ae C42:5	1.5 ± 0.3	0.04	(-0.06;0.14)	0.438	0.733	1.5 ± 0.3	ref.	1.6 ± 0.3	0.12	(-0.23;0.47)	0.504	0.838	1.6 ± 0.3	0.18	(-0.17;0.53)	0.304	0.702	0.246	0.677
PC (acyl-alkyl) C44:3	PC ae C44:3	0.07 ± 0.02	0.00	(-0.10;0.10)	0.950	0.983	0.07 ± 0.02	ref.	0.07 ± 0.02	-0.16	(-0.49;0.18)	0.358	0.667	0.07 ± 0.02	0.03	(-0.31;0.37)	0.865	0.935	0.828	0.916
PC (acyl-alkyl) C44:4	PC ae C44:4	0.2 ± 0.1	0.08	(-0.03;0.18)	0.142	0.507	0.2 ± 0.1	ref.	0.2 ± 0.1	-0.07	(-0.41;0.28)	0.706	0.876	0.2 ± 0.1	0.24	(-0.11;0.59)	0.177	0.624	0.151	0.598
PC (acyl-alkyl) C44:5	PC ae C44:5	1.0 ± 0.3	0.02	(-0.09;0.12)	0.775	0.910	1.0 ± 0.3	ref.	1.0 ± 0.3	0.06	(-0.29;0.42)	0.722	0.878	1.0 ± 0.3	0.06	(-0.30;0.42)	0.744	0.910	0.674	0.886
PC (acyl-alkyl) C44:6	PC ae C44:6	0.8 ± 0.2	0.10	(0.00;0.20)	0.053	0.362	0.7 ± 0.2	ref.	0.8 ± 0.2	0.07	(-0.27;0.41)	0.678	0.876	0.8 ± 0.2	0.32	(-0.02;0.66)	0.068	0.539	0.053	0.447
SPHINGOLIPIDS																				
Hydroxysphingomyelin C14:1	SM OH C14:1	4.0 ± 1.0	0.06	(-0.03;0.15)	0.209	0.608	3.6 ± 0.9	ref.	4.2 ± 1.2	0.15	(-0.16;0.46)	0.341	0.643	4.2 ± 0.9	0.23	(-0.09;0.54)	0.158	0.607	0.128	0.598
Hydroxysphingomyelin C16:1	SM OH C16:1	1.9 ± 0.5	0.08	(-0.02;0.17)	0.119	0.469	1.7 ± 0.4	ref.	1.9 ± 0.5	0.25	(-0.07;0.57)	0.122	0.493	1.9 ± 0.5	0.29	(-0.04;0.61)	0.084	0.565	0.070	0.549

Hydroxysphingomyelin C22:1 SM OH	4.4 ± 1.1	0.05	(-0.05;0.15)	0.302	0.718	4.1 ± 1.0	ref.	4.5 ± 1.2	0.05	(-0.27;0.38)	0.749	0.895	4.6 ± 1.0	0.21	(-0.12;0.54)	0.216	0.674	0.162	0.598
Hydroxysphingomyelin C22:2 SM OH	3.3 ± 0.9	0.05	(-0.04;0.14)	0.252	0.637	3.0 ± 0.8	ref.	3.5 ± 1.0	0.18	(-0.13;0.49)	0.248	0.564	3.5 ± 0.8	0.28	(-0.03;0.59)	0.078	0.558	0.052	0.447
Hydroxysphingomyelin C24:1 SM	0.3 ± 0.1	0.06	(-0.04;0.17)	0.239	0.629	0.3 ± 0.1	ref.	0.3 ± 0.1	0.25	(-0.10;0.60)	0.161	0.514	0.3 ± 0.1	0.27	(-0.08;0.62)	0.129	0.578	0.091	0.555
Sphingomyelin C16:0 SM	67.2 ± 13.6	-0.01	(-0.10;0.09)	0.863	0.943	65.3 ± 14.5	ref.	67.9 ± 13.2	-0.09	(-0.41;0.23)	0.590	0.876	68.3 ± 13.1	-0.04	(-0.36;0.29)	0.832	0.935	0.939	0.979
Sphingomyelin C16:1 SM	9.5 ± 2.2	-0.02	(-0.11;0.07)	0.615	0.852	9.0 ± 2.0	ref.	9.7 ± 2.4	-0.07	(-0.38;0.23)	0.634	0.876	9.7 ± 2.1	-0.03	(-0.34;0.27)	0.824	0.935	0.975	0.985
Sphingomyelin C18:0 SM	12.0 ± 3.3	0.01	(-0.09;0.11)	0.853	0.943	11.7 ± 4.0	ref.	12.2 ± 3.1	-0.01	(-0.35;0.33)	0.949	0.967	12.2 ± 2.7	0.05	(-0.28;0.39)	0.753	0.910	0.703	0.897
Sphingomyelin C18:1 SM	5.7 ± 1.6	0.00	(-0.09;0.09)	0.976	0.983	5.4 ± 1.8	ref.	5.8 ± 1.7	0.01	(-0.31;0.33)	0.954	0.967	5.8 ± 1.5	0.06	(-0.26;0.37)	0.731	0.909	0.620	0.823
Sphingomyelin C20:2 SM	0.2 ± 0.1	0.03	(-0.07;0.12)	0.566	0.825	0.1 ± 0.1	ref.	0.2 ± 0.1	0.16	(-0.16;0.49)	0.324	0.629	0.15 ± 0.05	0.09	(-0.24;0.41)	0.607	0.847	0.535	0.804
Sphingomyelin C24:0 SM	6.1 ± 1.4	-0.01	(-0.11;0.09)	0.896	0.964	6.0 ± 1.4	ref.	6.1 ± 1.3	-0.13	(-0.47;0.21)	0.456	0.774	6.3 ± 1.4	0.03	(-0.32;0.37)	0.872	0.935	0.726	0.916
Sphingomyelin C24:1 SM	13.9 ± 3.4	0.00	(-0.10;0.10)	0.960	0.983	13.9 ± 4.4	ref.	13.8 ± 2.8	-0.04	(-0.39;0.30)	0.809	0.903	13.9 ± 2.9	0.02	(-0.33;0.36)	0.913	0.964	0.834	0.916
Sphingomyelin C26:0 SM	0.04 ± 0.01	0.02	(-0.09;0.12)	0.735	0.910	0.03 ± 0.01	ref.	0.04 ± 0.01	0.33	(-0.03;0.68)	0.074	0.430	0.04 ± 0.02	0.24	(-0.12;0.59)	0.192	0.659	0.131	0.598
Sphingomyelin C26:1 SM	0.10 ± 0.04	0.05	(-0.05;0.16)	0.338	0.718	0.1 ± 0.1	ref.	0.10 ± 0.03	0.08	(-0.28;0.44)	0.674	0.876	0.11 ± 0.03	0.22	(-0.14;0.58)	0.230	0.682	0.197	0.643
HEXOSES																			
Hexoses	5574.0 ± 1481.3	-0.03	(-0.13;0.08)	0.620	0.852	5590.4 ± 1221.1	ref.	5440.8 ± 1494.2	-0.28	(-0.64;0.08)	0.131	0.493	5690.8 ± 1701.5	-0.11	(-0.48;0.25)	0.547	0.815	0.488	0.794

Western pattern

Metabolite	Abbreviation	Continuous	Tertile 1		Tertile 2		Tertile 3		p_{trend}	p_{FDR}^4		
			Mean $\pm$ SD ^{2}	β	Mean $\pm$ SD ^{2}	β	Mean $\pm$ SD ^{2}	β			95% CI ^{3}	p -value
ACYLCARNITINES												
Carnitine	C0	-0.03	36.8 $\pm$ 7.7	ref.	35.4 $\pm$ 7.3	-0.18	(-0.54;0.17)	0.313	0.969	0.543	0.261	0.514
Acetylcarnitine	C2	0.02	6.2 $\pm$ 2.0	ref.	6.0 $\pm$ 1.7	-0.07	(-0.42;0.27)	0.675	0.969	0.386	0.202	0.424
Propionylcarnitine	C3	-0.03	0.5 $\pm$ 0.2	ref.	0.5 $\pm$ 0.2	-0.05	(-0.40;0.30)	0.790	0.969	0.824	0.707	0.839
Butyrylcarnitine	C4	0.07	0.2 $\pm$ 0.1	ref.	0.2 $\pm$ 0.1	0.01	(-0.34;0.37)	0.955	0.997	0.944	0.889	0.943
Valeryl carnitine	C5	0.06	0.2 $\pm$ 0.1	ref.	0.2 $\pm$ 0.1	-0.02	(-0.35;0.31)	0.905	0.997	0.630	0.389	0.652
Decenylcarnitine	C10:1	0.02	0.1 $\pm$ 0.1	ref.	0.1 $\pm$ 0.1	-0.25	(-0.60;0.11)	0.173	0.969	0.644	0.347	0.598
Tetradecenylcarnitine	C14:1	0.12	0.06 $\pm$ 0.03	ref.	0.05 $\pm$ 0.02	-0.06	(-0.40;0.29)	0.750	0.969	0.926	0.901	0.943
Tetradecadienylcarnitine	C14:2	0.05	0.03 $\pm$ 0.01	ref.	0.02 $\pm$ 0.01	-0.13	(-0.48;0.21)	0.454	0.969	0.804	0.622	0.806
Hexadecanoylcarnitine	C16	0.10	0.11 $\pm$ 0.03	ref.	0.10 $\pm$ 0.02	-0.13	(-0.47;0.22)	0.469	0.969	0.640	0.489	0.730
Octadecanoylcarnitine	C18	0.15	0.05 $\pm$ 0.01	ref.	0.05 $\pm$ 0.01	0.01	(-0.34;0.36)	0.951	0.997	0.386	0.210	0.428
Octadecenylcarnitine	C18:1	-0.03	0.09 $\pm$ 0.04	ref.	0.08 $\pm$ 0.02	-0.20	(-0.56;0.16)	0.279	0.969	0.731	0.469	0.714
Octadecadienylcarnitine	C18:2	-0.15	0.04 $\pm$ 0.02	ref.	0.03 $\pm$ 0.01	-0.29	(-0.65;0.07)	0.116	0.969	0.386	0.143	0.392
AMINO ACIDS												
Alanine	Ala	0.01	385.3 $\pm$ 93.4	ref.	380.1 $\pm$ 108.3	0.00	(-0.36;0.36)	0.990	0.997	0.971	0.966	0.973
Arginine	Arg	0.11	78.9 $\pm$ 22.3	ref.	85.1 $\pm$ 23.1	0.27	(-0.08;0.62)	0.137	0.969	0.712	0.412	0.674
Asparagine	Asn	0.02	42.9 $\pm$ 8.3	ref.	44.9 $\pm$ 9.4	0.24	(-0.11;0.58)	0.178	0.969	0.971	0.838	0.913
Aspartate	Asp	-0.01	11.8 $\pm$ 3.8	ref.	11.8 $\pm$ 4.2	-0.02	(-0.38;0.34)	0.922	0.997	0.792	0.669	0.815
Citrulline	Cit	-0.01	36.4 $\pm$ 10.2	ref.	37.0 $\pm$ 10.2	0.16	(-0.20;0.51)	0.394	0.969	0.792	0.587	0.795

Glutamine	Gln	696.5 ± 98.5	0.08	(-0.06;0.23)	0.255	0.437	692.5 ± 86.4	ref.	703.0 ± 102.3	0.05	(-0.29;0.38)	0.788	0.969	694.1 ± 106.8	-0.01	(-0.37;-0.34)	0.937	0.971	0.987	0.987
Glutamate	Glu	80.0 ± 29.7	0.09	(-0.06;0.24)	0.241	0.430	80.1 ± 31	ref.	77.9 ± 30.0	0.05	(-0.31;0.40)	0.798	0.969	81.9 ± 28.4	0.18	(-0.19;0.54)	0.346	0.603	0.348	0.598
Glycine	Gly	215.6 ± 59.8	-0.12	(-0.26;0.02)	0.096	0.218	221.4 ± 65.2	ref.	220.6 ± 61.6	-0.07	(-0.39;0.26)	0.681	0.969	204.8 ± 51.1	-0.27	(-0.61;-0.07)	0.120	0.335	0.159	0.392
Histidine	His	71.1 ± 12.4	0.16	(0.01;-0.31)	0.035	0.107	69.5 ± 11.4	ref.	70.9 ± 13.4	0.08	(-0.27;0.43)	0.648	0.969	73.0 ± 12.3	0.19	(-0.17;0.55)	0.300	0.553	0.306	0.561
Isoleucine	Ile	75.4 ± 23.9	-0.08	(-0.22;0.07)	0.296	0.490	75.3 ± 22.4	ref.	79.1 ± 27.3	0.16	(-0.18;0.50)	0.356	0.969	71.7 ± 21.6	-0.15	(-0.51;-0.20)	0.392	0.641	0.496	0.730
Leucine	Leu	133.8 ± 42.3	0.02	(-0.13;0.16)	0.839	0.892	131.6 ± 40.3	ref.	139.4 ± 47.2	0.21	(-0.14;0.55)	0.241	0.969	130.4 ± 39	0.00	(-0.36;0.35)	0.979	0.979	0.892	0.943
Lysine	Lys	193.5 ± 41.8	0.05	(-0.09;0.20)	0.471	0.625	191.6 ± 34.0	ref.	199.4 ± 43.1	0.17	(-0.18;0.51)	0.339	0.969	189.5 ± 47.1	-0.09	(-0.45;0.27)	0.629	0.792	0.749	0.858
Methionine	Met	20.20 ± 5.8	-0.02	(-0.17;0.13)	0.787	0.886	20.8 ± 5.8	ref.	20.4 ± 6.0	0.03	(-0.31;0.38)	0.856	0.997	19.5 ± 5.6	-0.12	(-0.48;0.24)	0.498	0.712	0.506	0.730
Ornithine	Orn	71.3 ± 24.6	-0.07	(-0.22;0.08)	0.366	0.521	72.3 ± 22.3	ref.	70.6 ± 22.8	-0.07	(-0.43;0.28)	0.680	0.969	71.1 ± 28.5	-0.05	(-0.42;0.32)	0.777	0.874	0.747	0.858
Phenylalanine	Phe	62.8 ± 13.6	-0.02	(-0.16;0.13)	0.832	0.892	63.8 ± 13.0	ref.	63.8 ± 14.4	0.07	(-0.27;0.41)	0.684	0.969	60.7 ± 13.4	-0.12	(-0.47;0.24)	0.521	0.720	0.555	0.761
Proline	Pro	242.6 ± 79.5	-0.07	(-0.22;0.08)	0.344	0.512	246.5 ± 70.8	ref.	253.1 ± 86.6	0.05	(-0.30;0.39)	0.788	0.969	228.2 ± 79.4	-0.21	(-0.56;0.15)	0.257	0.500	0.334	0.597
Serine	Ser	93 ± 20.3	-0.02	(-0.17;0.14)	0.832	0.892	92.5 ± 19.6	ref.	95.5 ± 17.1	0.24	(-0.11;0.60)	0.176	0.969	91.0 ± 23.7	-0.01	(-0.38;0.36)	0.952	0.971	0.929	0.952
Threonine	Thr	108.7 ± 27.7	-0.08	(-0.22;0.07)	0.320	0.505	109.2 ± 24.0	ref.	110.2 ± 28.3	0.09	(-0.26;0.43)	0.624	0.969	106.8 ± 30.7	-0.12	(-0.49;0.24)	0.503	0.712	0.550	0.761
Tryptophan	Trp	53.1 ± 11.0	0.01	(-0.14;0.16)	0.890	0.924	54.2 ± 12.3	ref.	51.9 ± 11.6	-0.17	(-0.52;0.18)	0.341	0.969	53.2 ± 8.8	-0.07	(-0.44;-0.29)	0.699	0.819	0.640	0.806
Tyrosine	Tyr	74.8 ± 24.1	-0.01	(-0.16;0.14)	0.897	0.925	77.2 ± 26.5	ref.	75.7 ± 25.1	0.08	(-0.28;0.43)	0.672	0.969	71.3 ± 20.2	-0.02	(-0.39;0.34)	0.901	0.951	0.930	0.952
Valine	Val	242.1 ± 64.0	0.02	(-0.13;0.16)	0.826	0.892	241.6 ± 62	ref.	247.9 ± 75.2	0.09	(-0.25;0.43)	0.598	0.969	236.7 ± 53.6	-0.07	(-0.43;-0.29)	0.703	0.819	0.769	0.873
BIOGENIC AMINES																				
Creatinine	Creatinin e	74.5 ± 17.4	0.04	(-0.08;0.16)	0.542	0.683	74.8 ± 19.3	ref.	75.1 ± 18.2	0.06	(-0.22;0.35)	0.673	0.969	73.5 ± 14.4	-0.03	(-0.33;-0.27)	0.850	0.926	0.928	0.952
Kynurenine	Kynureni ne	2.7 ± 0.7	-0.07	(-0.21;0.08)	0.364	0.521	2.9 ± 0.8	ref.	2.5 ± 0.6	-0.38	(-0.71;- 0.05)	0.025	0.969	2.7 ± 0.8	-0.16	(-0.51;-0.18)	0.360	0.619	0.220	0.440
Sarcosine	Sarcosine	4.4 ± 1.1	0.05	(-0.11;0.20)	0.555	0.683	4.4 ± 1.1	ref.	4.3 ± 1.0	-0.08	(-0.43;0.27)	0.662	0.969	4.5 ± 1.2	0.09	(-0.28;0.46)	0.632	0.792	0.638	0.806
Serotonin	Serotonin	0.8 ± 0.6	0.06	(-0.10;0.21)	0.470	0.625	0.7 ± 0.5	ref.	0.7 ± 0.6	-0.02	(-0.37;0.34)	0.927	0.997	0.8 ± 0.5	0.12	(-0.25;-0.48)	0.540	0.731	0.625	0.806
Taurine	Taurine	120.9 ± 49.4	0.07	(-0.08;0.23)	0.346	0.512	120.6 ± 47.0	ref.	121.4 ± 51.6	0.00	(-0.36;0.36)	0.985	0.997	120.6 ± 50.3	0.04	(-0.34;-0.41)	0.849	0.926	0.868	0.938

Trans 4- Hydroxyproline Asymmetric dimethylarginine	14-OH- Pro	10.2 ± 5.0	0.03	(-0.12;0.18)	0.724	0.843	10.6 ± 6.1	ref.	10.3 ± 4.6	-0.01	(-0.35;0.34)	0.967	0.997	9.6 ± 4.2	-0.12	(-0.48;0.24)	0.505	0.712	0.557	0.761
Symmetric dimethylarginine	ADMA	0.5 ± 0.1	-0.14	(-0.27;0.00)	0.046	0.130	0.5 ± 0.1	ref.	0.5 ± 0.1	0.00	(-0.32;0.31)	0.995	0.997	0.4 ± 0.1	-0.31	(-0.64;0.02)	0.068	0.238	0.104	0.316
	SDMA	0.5 ± 0.1	0.06	(-0.08;0.20)	0.417	0.570	0.5 ± 0.1	ref.	0.5 ± 0.1	0.11	(-0.22;0.43)	0.525	0.969	0.4 ± 0.1	-0.12	(-0.47;0.22)	0.476	0.708	0.601	0.797
GLYCEROPHOSPHOLIPIDS																				
	LysoPC (acyl) C16:0	90.4 ± 20.1	0.05	(-0.11;0.20)	0.553	0.683	88.6 ± 19.6	ref.	89.8 ± 20.5	-0.05	(-0.41;0.32)	0.802	0.969	92.8 ± 20.2	0.10	(-0.28;0.48)	0.605	0.792	0.596	0.797
	LysoPC (acyl) C16:1	2.6 ± 0.9	0.09	(-0.07;0.24)	0.271	0.459	2.5 ± 0.9	ref.	2.6 ± 1.1	0.01	(-0.36;0.37)	0.976	0.997	2.6 ± 0.8	0.15	(-0.22;0.53)	0.432	0.673	0.430	0.680
	LysoPC (acyl) C17:0	1.6 ± 0.5	0.07	(-0.08;0.22)	0.356	0.519	1.5 ± 0.4	ref.	1.6 ± 0.5	0.10	(-0.25;0.44)	0.573	0.969	1.6 ± 0.6	0.22	(-0.14;0.58)	0.225	0.469	0.200	0.424
	LysoPC (acyl) C18:0	26.4 ± 6.8	0.05	(-0.11;0.20)	0.550	0.683	25.5 ± 5.7	ref.	26.2 ± 6.6	-0.03	(-0.38;0.32)	0.858	0.997	27.4 ± 7.9	0.15	(-0.22;0.52)	0.426	0.671	0.422	0.680
	LysoPC (acyl) C18:1	17.9 ± 5.4	0.11	(-0.04;0.26)	0.139	0.292	16.9 ± 5.0	ref.	18.4 ± 5.7	0.16	(-0.20;0.51)	0.386	0.969	18.5 ± 5.3	0.23	(-0.13;0.60)	0.216	0.459	0.184	0.399
	LysoPC (acyl) C18:2	29.2 ± 10.4	0.05	(-0.10;0.20)	0.515	0.663	27.2 ± 9.6	ref.	30.4 ± 11	0.10	(-0.24;0.45)	0.558	0.969	30.20 ± 10.3	0.11	(-0.25;0.47)	0.551	0.739	0.468	0.714
	LysoPC (acyl) C20:3	2.0 ± 0.7	0.04	(-0.11;0.19)	0.591	0.720	1.9 ± 0.7	ref.	2.1 ± 0.7	0.06	(-0.29;0.41)	0.756	0.969	2.2 ± 0.7	0.14	(-0.22;0.51)	0.444	0.684	0.412	0.674
	LysoPC (acyl) C20:4	5.9 ± 1.7	0.00	(-0.15;0.16)	0.961	0.962	6.0 ± 2.0	ref.	5.8 ± 1.5	-0.15	(-0.51;0.20)	0.401	0.969	5.9 ± 1.6	-0.08	(-0.45;0.29)	0.663	0.804	0.667	0.815
	LysoPC (acyl) C24:0	0.14 ± 0.03	-0.05	(-0.19;0.09)	0.499	0.656	0.14 ± 0.03	ref.	0.13 ± 0.03	-0.20	(-0.52;0.12)	0.220	0.969	0.14 ± 0.03	-0.03	(-0.36;0.31)	0.877	0.944	0.802	0.896
	LysoPC (acyl) C28:1	0.3 ± 0.1	0.08	(-0.04;0.21)	0.190	0.360	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.05	(-0.34;0.24)	0.735	0.969	0.3 ± 0.1	0.18	(-0.12;0.48)	0.245	0.489	0.283	0.534
	PC (diacyl) C28:1	3.0 ± 0.8	0.31	(0.18;0.44)	0.000	0.001	2.9 ± 0.8	ref.	3.0 ± 0.8	0.18	(-0.14;0.50)	0.274	0.969	3.1 ± 0.7	0.59	(0.26;0.92)	0.001	0.083	0.001	0.100
	PC (diacyl) C30:0	4.0 ± 1.3	0.23	(0.09;0.38)	0.002	0.016	3.8 ± 1.5	ref.	4.0 ± 1.3	0.22	(-0.13;0.57)	0.210	0.969	4.2 ± 1.2	0.47	(0.11;0.84)	0.012	0.095	0.013	0.108
	PC (diacyl) C32:0	26.6 ± 5.5	0.17	(0.02;0.31)	0.025	0.081	26.5 ± 5.6	ref.	26.8 ± 5.6	0.11	(-0.24;0.45)	0.545	0.969	26.6 ± 5.3	0.21	(-0.15;0.56)	0.257	0.500	0.266	0.516
	PC (diacyl) C32:1	32.0 ± 16.2	0.17	(0.02;0.33)	0.027	0.085	31.2 ± 17.3	ref.	31.4 ± 16	0.11	(-0.25;0.47)	0.543	0.969	33.5 ± 15.2	0.30	(-0.07;0.67)	0.114	0.333	0.123	0.351
	PC (diacyl) C32:2	8.3 ± 3.1	0.21	(0.06;0.36)	0.005	0.031	7.9 ± 3.5	ref.	8.3 ± 3.0	0.13	(-0.22;0.48)	0.467	0.969	8.9 ± 2.7	0.40	(0.04;0.77)	0.030	0.150	0.031	0.154
	PC (diacyl) C32:3	0.9 ± 0.2	0.12	(-0.02;0.26)	0.086	0.210	0.9 ± 0.3	ref.	0.9 ± 0.2	0.10	(-0.22;0.43)	0.528	0.969	0.9 ± 0.2	0.27	(-0.07;0.60)	0.121	0.335	0.117	0.340
	PC (diacyl) C34:1	36.5 ± 82.8	0.24	(0.09;0.38)	0.002	0.016	352.8 ± 83.8	ref.	368.1 ± 82.1	0.22	(-0.13;0.57)	0.218	0.969	375.3 ± 82.3	0.40	(0.04;0.77)	0.033	0.153	0.032	0.154

PC (diacyl) C34:2	PC aa C34:2	739.8 ± 128.1	0.20	(0.05;0.35)	0.009	0.036	718.4 ± 135.0	ref.	747.2 ± 123.7	0.19	(-0.17;0.54)	0.307	0.969	753.7 ± 124.4	0.32	(-0.06;0.69)	0.097	0.304	0.086	0.295
PC (diacyl) C34:3	PC aa C34:3	30.1 ± 10.2	0.15	(0.00;0.29)	0.052	0.143	10.7	ref.	30.6 ± 10.8	0.12	(-0.23;0.46)	0.513	0.969	30.8 ± 8.9	0.27	(-0.09;0.63)	0.150	0.374	0.140	0.391
PC (diacyl) C34:4	PC aa C34:4	3.1 ± 1.2	0.19	(0.04;0.33)	0.014	0.050	3.0 ± 1.4	ref.	3.1 ± 1.1	0.10	(-0.25;0.44)	0.577	0.969	3.3 ± 1.0	0.36	(0.00;0.72)	0.050	0.197	0.051	0.209
PC (diacyl) C36:0	PC aa C36:0	3.2 ± 1.0	0.10	(-0.04;0.25)	0.168	0.335	3.1 ± 0.9	ref.	3.3 ± 1.1	0.10	(-0.24;0.44)	0.572	0.969	3.2 ± 1.1	0.24	(-0.11;0.60)	0.179	0.386	0.173	0.392
PC (diacyl) C36:1	PC aa C36:1	62.9 ± 17.8	0.24	(0.1;0.38)	0.001	0.010	58.7 ± 17.1	ref.	63.3 ± 17.6	0.24	(-0.08;0.57)	0.146	0.969	66.9 ± 17.9	0.54	(0.2;0.89)	0.002	0.089	0.002	0.100
PC (diacyl) C36:2	PC aa C36:2	384.7 ± 79.7	0.19	(0.05;0.33)	0.009	0.036	366.3 ± 73.4	ref.	390.7 ± 80.5	0.22	(-0.12;0.55)	0.202	0.969	397.3 ± 82.7	0.38	(0.03;0.73)	0.033	0.153	0.028	0.149
PC (diacyl) C36:3	PC aa C36:3	192.6 ± 43.8	0.21	(0.07;0.35)	0.004	0.024	183 ± 44.8	ref.	193.0 ± 41.1	0.18	(-0.15;0.51)	0.289	0.969	201.9 ± 44	0.41	(0.06;0.76)	0.021	0.119	0.021	0.126
PC (diacyl) C36:4	PC aa C36:4	291.5 ± 72.0	0.17	(0.02;0.31)	0.023	0.078	297.2 ± 89.5	ref.	285.7 ± 61	-0.08	(-0.42;0.26)	0.635	0.969	291.5 ± 62.6	0.12	(-0.23;0.48)	0.493	0.712	0.529	0.754
PC (diacyl) C36:5	PC aa C36:5	45.8 ± 27.8	0.00	(-0.15;0.16)	0.962	0.962	44.4 ± 21.2	ref.	47.1 ± 34.4	-0.01	(-0.36;0.34)	0.954	0.997	45.7 ± 26.5	0.08	(-0.28;0.45)	0.657	0.804	0.682	0.824
PC (diacyl) C36:6	PC aa C36:6	1.5 ± 0.6	0.10	(-0.05;0.25)	0.196	0.360	1.5 ± 0.6	ref.	1.6 ± 0.7	0.14	(-0.20;0.48)	0.426	0.969	1.6 ± 0.6	0.30	(-0.06;0.66)	0.103	0.315	0.093	0.310
PC (diacyl) C38:0	PC aa C38:0	2.9 ± 0.9	0.08	(-0.07;0.22)	0.288	0.482	2.8 ± 0.9	ref.	2.9 ± 0.9	0.05	(-0.28;0.39)	0.759	0.969	2.9 ± 0.9	0.26	(-0.09;0.61)	0.141	0.371	0.162	0.392
PC (diacyl) C38:3	PC aa C38:3	53.2 ± 13.6	0.12	(-0.02;0.25)	0.097	0.218	51.5 ± 13.8	ref.	52.2 ± 12.4	0.06	(-0.26;0.38)	0.719	0.969	55.9 ± 14.4	0.34	(0.01;0.68)	0.042	0.183	0.051	0.209
PC (diacyl) C38:4	PC aa C38:4	131.5 ± 35.0	0.10	(-0.03;0.24)	0.140	0.292	136.2 ± 42.3	ref.	128.0 ± 29.9	-0.14	(-0.46;0.18)	0.385	0.969	130.4 ± 31.5	0.06	(-0.27;0.39)	0.733	0.832	0.807	0.896
PC (diacyl) C38:5	PC aa C38:5	66.2 ± 19.0	0.07	(-0.07;0.21)	0.328	0.511	66.7 ± 19.1	ref.	66.0 ± 20.8	-0.08	(-0.41;0.25)	0.645	0.969	66.0 ± 17.3	0.07	(-0.27;0.42)	0.678	0.804	0.726	0.846
PC (diacyl) C38:6	PC aa C38:6	101.1 ± 33.6	0.02	(-0.13;0.17)	0.793	0.886	102.1 ± 29.9	ref.	101.1 ± 37.8	-0.08	(-0.42;0.27)	0.663	0.969	100.2 ± 33.1	0.10	(-0.26;0.45)	0.603	0.792	0.630	0.806
PC (diacyl) C40:2	PC aa C40:2	0.3 ± 0.1	0.09	(-0.06;0.23)	0.244	0.430	0.3 ± 0.1	ref.	0.3 ± 0.1	0.13	(-0.21;0.46)	0.458	0.969	0.3 ± 0.1	0.24	(-0.11;0.59)	0.177	0.386	0.176	0.392
PC (diacyl) C40:3	PC aa C40:3	0.4 ± 0.1	0.07	(-0.07;0.21)	0.332	0.511	0.4 ± 0.1	ref.	0.4 ± 0.2	0.17	(-0.16;0.50)	0.326	0.969	0.4 ± 0.1	0.21	(-0.13;0.56)	0.228	0.469	0.211	0.428
PC (diacyl) C40:4	PC aa C40:4	3.0 ± 0.8	0.19	(0.05;0.34)	0.011	0.041	2.9 ± 0.8	ref.	2.9 ± 0.8	-0.02	(-0.36;0.33)	0.924	0.997	3.2 ± 0.9	0.28	(-0.08;0.65)	0.124	0.335	0.153	0.392
PC (diacyl) C40:5	PC aa C40:5	8.3 ± 2.4	0.10	(-0.05;0.25)	0.191	0.360	8.3 ± 2.4	ref.	8.0 ± 2.4	-0.11	(-0.45;0.23)	0.529	0.969	8.5 ± 2.6	0.13	(-0.22;0.49)	0.463	0.701	0.546	0.761
PC (diacyl) C40:6	PC aa C40:6	28.3 ± 10.2	-0.02	(-0.17;0.12)	0.775	0.886	29.2 ± 9.8	ref.	27.8 ± 10.5	-0.09	(-0.43;0.24)	0.590	0.969	27.9 ± 10.4	0.08	(-0.27;0.43)	0.659	0.804	0.717	0.843
PC (diacyl) C42:0	PC aa C42:0	0.3 ± 0.1	-0.05	(-0.19;0.09)	0.512	0.663	0.4 ± 0.1	ref.	0.3 ± 0.1	-0.09	(-0.42;0.24)	0.595	0.969	0.3 ± 0.1	-0.01	(-0.36;0.33)	0.935	0.971	0.899	0.943
PC (diacyl) C42:1	PC aa C42:1	0.18 ± 0.05	0.01	(-0.13;0.15)	0.920	0.941	0.2 ± 0.1	ref.	0.18 ± 0.05	-0.02	(-0.35;0.31)	0.909	0.997	0.18 ± 0.05	0.09	(-0.26;0.43)	0.625	0.792	0.666	0.815

PC (diacyl) C42:2	PC aa C42:2	0.16 ± 0.04	0.18	(-0.17;-0.54)	0.310	0.553	0.315	0.571
PC (diacyl) C42:4	PC aa C42:4	0.10 ± 0.03	0.04	(-0.33;-0.41)	0.840	0.926	0.834	0.913
PC (diacyl) C42:5	PC aa C42:5	0.3 ± 0.1	0.21	(-0.16;-0.58)	0.268	0.513	0.296	0.550
PC (diacyl) C42:6	PC aa C42:6	0.4 ± 0.1	0.15	(-0.21;-0.52)	0.404	0.644	0.449	0.699
PC (acyl-alkyl) C30:0	PC ae C30:0	0.4 ± 0.1	0.49	(0.15;0.84)	0.006	0.089	0.005	0.100
PC (acyl-alkyl) C30:2	PC ae C30:2	0.08 ± 0.02	0.29	(-0.02;-0.59)	0.069	0.238	0.084	0.295
PC (acyl-alkyl) C32:1	PC ae C32:1	5.3 ± 1.2	0.36	(0.01;-0.71)	0.047	0.192	0.049	0.209
PC (acyl-alkyl) C32:2	PC ae C32:2	1.4 ± 0.4	0.33	(-0.01;-0.67)	0.057	0.214	0.054	0.209
PC (acyl-alkyl) C34:0	PC ae C34:0	2.5 ± 0.6	0.50	(0.15;0.85)	0.006	0.089	0.006	0.100
PC (acyl-alkyl) C34:1	PC ae C34:1	18.0 ± 3.9	0.44	(0.11;-0.78)	0.010	0.094	0.010	0.108
PC (acyl-alkyl) C34:2	PC ae C34:2	20.2 ± 5.3	0.44	(0.09;0.78)	0.013	0.100	0.012	0.108
PC (acyl-alkyl) C34:3	PC ae C34:3	13.1 ± 4.0	0.42	(0.07;-0.76)	0.020	0.119	0.018	0.121
PC (acyl-alkyl) C36:0	PC ae C36:0	1.2 ± 0.3	0.48	(0.12;-0.84)	0.010	0.094	0.012	0.108
PC (acyl-alkyl) C36:1	PC ae C36:1	11.5 ± 2.7	0.51	(0.18;0.85)	0.003	0.089	0.003	0.100
PC (acyl-alkyl) C36:2	PC ae C36:2	21.7 ± 5.4	0.41	(0.08;0.74)	0.015	0.104	0.011	0.108
PC (acyl-alkyl) C36:3	PC ae C36:3	11.4 ± 2.8	0.45	(0.11;-0.79)	0.010	0.094	0.009	0.105
PC (acyl-alkyl) C36:4	PC ae C36:4	26.3 ± 6.4	0.40	(0.04;-0.76)	0.029	0.150	0.032	0.154
PC (acyl-alkyl) C36:5	PC ae C36:5	18.3 ± 5.0	0.26	(-0.09;-0.61)	0.146	0.374	0.155	0.392
PC (acyl-alkyl) C38:0	PC ae C38:0	2.5 ± 0.8	0.19	(-0.17;-0.54)	0.302	0.553	0.282	0.534
PC (acyl-alkyl) C38:2	PC ae C38:2	2.2 ± 0.6	0.47	(0.13;0.81)	0.008	0.094	0.006	0.100
PC (acyl-alkyl) C38:3	PC ae C38:3	4.8 ± 1.1	0.49	(0.16;0.81)	0.004	0.089	0.004	0.100
PC (acyl-alkyl) C38:4	PC ae C38:4	17.2 ± 3.6	0.45	(0.11;-0.79)	0.011	0.094	0.015	0.112

PC (ae C38:5)	21.9 ± 4.7	0.19	(0.05;0.33)	0.009	0.036	21.5 ± 4.8	ref.	22.2 ± 4.6	0.10	(-0.23;0.44)	0.550	0.969	22.1 ± 4.6	0.30	(-0.05;0.65)	0.090	0.302	0.099	0.316
PC (ae C38:6)	9.2 ± 2.6	0.13	(-0.01;0.27)	0.068	0.174	9.0 ± 2.3	ref.	9.4 ± 2.8	0.08	(-0.25;0.41)	0.639	0.969	9.4 ± 2.8	0.30	(-0.05;0.64)	0.093	0.303	0.104	0.316
PC (ae C40:1)	1.1 ± 0.3	0.08	(-0.07;0.22)	0.316	0.505	1.1 ± 0.3	ref.	1.1 ± 0.3	-0.09	(-0.43;0.25)	0.606	0.969	1.1 ± 0.3	0.13	(-0.22;0.49)	0.466	0.701	0.503	0.730
PC (ae C40:2)	1.6 ± 0.4	0.23	(0.1;0.37)	0.001	0.010	1.5 ± 0.4	ref.	1.5 ± 0.4	0.06	(-0.27;0.38)	0.736	0.969	1.6 ± 0.4	0.50	(0.16;0.83)	0.004	0.089	0.007	0.100
PC (ae C40:3)	0.8 ± 0.2	0.17	(0.04;0.30)	0.013	0.047	0.8 ± 0.2	ref.	0.8 ± 0.2	0.11	(-0.20;0.42)	0.475	0.969	0.9 ± 0.2	0.42	(0.1;0.74)	0.011	0.094	0.014	0.108
PC (ae C40:4)	2.2 ± 0.4	0.23	(0.09;0.37)	0.001	0.010	2.2 ± 0.5	ref.	2.2 ± 0.5	0.07	(-0.25;0.40)	0.664	0.969	2.3 ± 0.4	0.42	(0.08;0.76)	0.017	0.108	0.024	0.137
PC (ae C40:5)	3.4 ± 0.7	0.19	(0.05;0.33)	0.009	0.036	3.3 ± 0.7	ref.	3.3 ± 0.7	-0.02	(-0.34;0.30)	0.896	0.997	3.5 ± 0.6	0.39	(0.05;0.72)	0.025	0.136	0.040	0.179
PC (ae C40:6)	4.8 ± 1.2	0.07	(-0.07;0.21)	0.311	0.505	4.8 ± 1.2	ref.	4.8 ± 1.2	-0.02	(-0.34;0.31)	0.927	0.997	4.8 ± 1.3	0.25	(-0.09;0.59)	0.154	0.374	0.180	0.395
PC (ae C42:1)	0.3 ± 0.1	0.07	(-0.07;0.21)	0.348	0.512	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.01	(-0.34;0.32)	0.956	0.997	0.3 ± 0.1	0.06	(-0.28;0.41)	0.719	0.824	0.699	0.836
PC (ae C42:2)	0.4 ± 0.1	0.12	(-0.02;0.26)	0.092	0.217	0.4 ± 0.1	ref.	0.4 ± 0.1	0.07	(-0.25;0.39)	0.650	0.969	0.4 ± 0.1	0.34	(0.01;0.67)	0.047	0.192	0.055	0.209
PC (ae C42:3)	0.6 ± 0.1	0.13	(-0.01;0.27)	$\frac{0.078}{2}$	0.194	0.5 ± 0.2	ref.	0.6 ± 0.1	0.14	(-0.19;0.47)	$\frac{0.399}{2}$	0.969	0.6 ± 0.1	0.33	(-0.01;0.68)	0.0576	0.214	0.059	0.219
PC (ae C42:4)	0.7 ± 0.2	0.17	(0.02;0.31)	0.023	0.078	0.7 ± 0.2	ref.	0.7 ± 0.2	0.14	(-0.19;0.47)	0.417	0.969	0.7 ± 0.1	0.29	(-0.05;0.64)	0.097	0.304	0.097	0.316
PC (ae C42:5)	1.5 ± 0.3	0.13	(-0.01;0.28)	0.074	0.187	1.5 ± 0.4	ref.	1.5 ± 0.3	-0.06	(-0.39;0.27)	0.727	0.969	1.6 ± 0.3	0.27	(-0.07;0.62)	0.125	0.335	0.161	0.392
PC (ae C44:3)	0.07 ± 0.02	0.03	(-0.11;0.17)	0.684	0.815	0.07 ± 0.02	ref.	0.07 ± 0.02	0.05	(-0.27;0.38)	0.748	0.969	0.07 ± 0.02	-0.01	(-0.35;0.33)	0.951	0.971	0.953	0.967
PC (ae C44:4)	0.2 ± 0.1	0.10	(-0.04;0.24)	0.179	0.352	0.2 ± 0.1	ref.	0.2 ± 0.1	-0.15	(-0.48;0.19)	0.390	0.969	0.23 ± 0.05	0.17	(-0.18;0.52)	0.342	0.603	0.431	0.680
PC (ae C44:5)	1.0 ± 0.3	0.13	(-0.02;0.28)	0.092	0.217	1.0 ± 0.3	ref.	1.0 ± 0.3	-0.06	(-0.41;0.28)	0.725	0.969	1.0 ± 0.2	0.19	(-0.17;0.55)	0.307	0.553	0.364	0.618
PC (ae C44:6)	0.8 ± 0.2	-0.01	(-0.16;0.13)	0.837	0.892	0.8 ± 0.2	ref.	0.8 ± 0.2	-0.20	(-0.53;0.13)	0.242	0.969	0.8 ± 0.2	-0.02	(-0.37;0.32)	0.887	0.944	0.809	0.896
SPHINGOLIPIDS																			
Hydroxysphingomyelin C14:1	4.0 ± 1.0	0.22	(0.09;0.34)	0.001	0.010	3.9 ± 1.0	ref.	4.0 ± 1.1	0.11	(-0.18;0.41)	0.462	0.969	4.1 ± 1.0	0.44	(0.13;0.75)	0.006	0.089	0.007	0.100
Hydroxysphingomyelin C16:1	1.9 ± 0.5	0.18	(0.05;0.31)	0.009	0.036	1.8 ± 0.5	ref.	1.9 ± 0.5	0.10	(-0.21;0.41)	0.542	0.969	1.9 ± 0.5	0.38	(0.06;0.71)	0.021	0.119	0.026	0.144
Hydroxysphingomyelin C22:1	4.4 ± 1.1	0.22	(0.09;0.36)	0.001	0.010	4.2 ± 0.9	ref.	4.3 ± 1.2	0.02	(-0.29;0.33)	0.901	0.997	4.7 ± 1.1	0.43	(0.11;0.76)	0.010	0.094	0.016	0.112

Hydroxysphingomyelin C22:2	SM OH	3.3 ± 0.9	0.15	(0.03;0.28)	0.019	0.067	3.2 ± 0.8	ref.	3.3 ± 1.0	0.05	(-0.25;0.35)	0.728	0.969	3.4 ± 0.9	0.30	(-0.02;0.61)	0.064	0.233	0.071	0.257
Hydroxysphingomyelin C24:1	SM OH	0.3 ± 0.1	0.28	(0.14;0.42)	0.000	0.010	0.3 ± 0.1	ref.	0.3 ± 0.1	0.06	(-0.27;0.39)	0.715	0.969	0.3 ± 0.1	0.52	(0.18;0.87)	0.003	0.089	0.005	0.100
Sphingomyelin C16:0	SM	67.2 ± 13.6	0.17	(0.04;0.31)	0.011	0.041	65.4 ± 12.3	ref.	67.5 ± 13.3	0.09	(-0.23;0.40)	0.591	0.969	68.6 ± 15.1	0.35	(0.03;0.68)	0.035	0.156	0.038	0.174
Sphingomyelin C16:1	SM	9.5 ± 2.2	0.11	(-0.02;0.23)	0.098	0.218	9.3 ± 2.0	ref.	9.6 ± 2.4	0.05	(-0.25;0.35)	0.741	0.969	9.5 ± 2.2	0.21	(-0.09;0.52)	0.176	0.386	0.167	0.392
Sphingomyelin C18:0	SM	12.0 ± 3.3	0.14	(0.00;0.28)	0.044	0.125	11.9 ± 3.1	ref.	12 ± 3.2	0.04	(-0.28;0.37)	0.793	0.969	12.2 ± 3.7	0.25	(-0.09;0.59)	0.153	0.374	0.170	0.392
Sphingomyelin C18:1	SM	5.7 ± 1.6	0.05	(-0.08;0.18)	0.443	0.599	5.7 ± 1.6	ref.	5.8 ± 1.7	0.03	(-0.28;0.34)	0.851	0.997	5.6 ± 1.6	0.07	(-0.25;0.39)	0.677	0.804	0.643	0.806
Sphingomyelin C20:2	SM	0.2 ± 0.1	-0.07	(-0.20;0.07)	0.320	0.505	0.16 ± 0.05	ref.	0.1 ± 0.1	-0.41	(-0.72;-0.10)	0.011	0.969	0.1 ± 0.1	-0.20	(-0.52;0.13)	0.231	0.469	0.166	0.392
Sphingomyelin C24:0	SM	6.1 ± 1.4	0.23	(0.09;0.37)	0.001	0.010	5.7 ± 1.3	ref.	6.0 ± 1.5	0.05	(-0.28;0.38)	0.778	0.969	6.6 ± 1.4	0.43	(0.08;0.77)	0.015	0.104	0.020	0.126
Sphingomyelin C24:1	SM	13.9 ± 3.4	0.16	(0.02;0.30)	0.031	0.097	13.3 ± 2.8	ref.	14.0 ± 3.8	0.09	(-0.25;0.42)	0.611	0.969	14.2 ± 3.6	0.24	(-0.10;0.59)	0.169	0.386	0.162	0.392
Sphingomyelin C26:0	SM	0.04 ± 0.01	0.16	(0.01;0.31)	0.036	0.107	0.04 ± 0.01	ref.	0.04 ± 0.01	0.10	(-0.24;0.45)	0.556	0.969	0.04 ± 0.01	0.16	(-0.20;0.52)	0.392	0.641	0.339	0.598
Sphingomyelin C26:1	SM	0.10 ± 0.04	0.11	(-0.04;0.26)	0.150	0.310	0.10 ± 0.03	ref.	0.1 ± 0.1	0.14	(-0.21;0.49)	0.424	0.969	0.11 ± 0.03	0.29	(-0.07;0.66)	0.114	0.333	0.109	0.323
HEXOSES																				
Hexoses	H1	5574.0 ± 1481.3	0.06	(-0.09;0.21)	0.413	0.570	5503.9 ± 1166.4	ref.	5612.4 ± 1481.7	0.11	(-0.24;0.47)	0.533	0.969	5605.5 ± 1757.1	0.12	(-0.25;0.49)	0.514	0.718	0.500	0.730

Carnivore pattern

Metabolite	Abbreviation	Mean $\pm$ SD ²	Continuous			Tertile 1		Tertile 2		Tertile 3			p_{trend}	p_{FDR}^4	
			β	95% CI ³	p -value	Mean $\pm$ SD ²	β	95% CI ³	p -value	Mean $\pm$ SD ²	β	95% CI ³			p -value
ACYLCARNITINES															
Carnitine	C0	36.0 $\pm$ 7.3	0.09	(-0.06;0.24)	0.227	0.584	34.4 $\pm$ 7.7	ref.	35.9 $\pm$ 6.8	0.17	(-0.19;0.52)	0.356	0.915	0.127	0.169
Acetylcarnitine	C2	6.0 $\pm$ 1.9	0.02	(-0.13;0.16)	0.824	0.917	5.7 $\pm$ 1.8	ref.	6.0 $\pm$ 1.7	0.06	(-0.29;0.41)	0.744	0.961	0.812	0.872
Propionylcarnitine	C3	0.5 $\pm$ 0.2	0.09	(-0.06;0.23)	0.237	0.599	0.4 $\pm$ 0.1	ref.	0.4 $\pm$ 0.1	0.00	(-0.34;0.35)	0.985	0.988	0.116	0.185
Butyrylcarnitine	C4	0.2 $\pm$ 0.1	0.09	(-0.06;0.24)	0.225	0.584	0.2 $\pm$ 0.1	ref.	0.2 $\pm$ 0.1	0.17	(-0.19;0.52)	0.359	0.915	0.306	0.316
Valeryl carnitine	C5	0.2 $\pm$ 0.1	0.15	(0.02;0.29)	0.027	0.170	0.1 $\pm$ 0.1	ref.	0.2 $\pm$ 0.1	0.27	(-0.05;0.60)	0.099	0.915	0.062	0.104
Decenoylcarnitine	C10:1	0.1 $\pm$ 0.1	0.07	(-0.08;0.21)	0.380	0.766	0.12 $\pm$ 0.05	ref.	0.13 $\pm$ 0.05	0.04	(-0.32;0.40)	0.827	0.961	0.888	0.872
Tetradecenoylcarnitine	C14:1	0.05 $\pm$ 0.02	-0.03	(-0.17;0.12)	0.730	0.901	0.05 $\pm$ 0.03	ref.	0.05 $\pm$ 0.02	-0.03	(-0.38;0.32)	0.845	0.961	0.801	0.869
Tetradecadienyl carnitine	C14:2	0.02 $\pm$ 0.01	-0.04	(-0.18;0.10)	0.577	0.901	0.02 $\pm$ 0.01	ref.	0.02 $\pm$ 0.01	-0.13	(-0.47;0.22)	0.479	0.915	0.716	0.781
Hexadecanoylcarnitine	C16	0.10 $\pm$ 0.03	0.01	(-0.14;0.15)	0.900	0.942	0.10 $\pm$ 0.03	ref.	0.10 $\pm$ 0.02	-0.01	(-0.36;0.34)	0.956	0.988	0.801	0.869
Octadecanoylcarnitine	C18	0.05 $\pm$ 0.01	-0.01	(-0.16;0.13)	0.887	0.936	0.05 $\pm$ 0.01	ref.	0.05 $\pm$ 0.01	0.10	(-0.25;0.45)	0.589	0.915	0.849	0.876
Octadecenoylcarnitine	C18:1	0.09 $\pm$ 0.03	-0.03	(-0.18;0.12)	0.666	0.901	0.09 $\pm$ 0.04	ref.	0.08 $\pm$ 0.03	-0.05	(-0.41;0.31)	0.780	0.961	0.888	0.876
Octadecadienyl carnitine	C18:2	0.03 $\pm$ 0.01	0.03	(-0.12;0.18)	0.712	0.901	0.03 $\pm$ 0.02	ref.	0.04 $\pm$ 0.01	0.07	(-0.30;0.43)	0.724	0.961	0.974	0.884
AMINO ACIDS															
Alanine	Ala	382.8 $\pm$ 100.7	-0.05	(-0.19;0.10)	0.550	0.901	378.1 $\pm$ 102.4	ref.	381.4 $\pm$ 103.1	-0.04	(-0.40;0.32)	0.820	0.961	0.920	0.879
Arginine	Arg	81.3 $\pm$ 23.5	0.13	(-0.02;0.27)	0.085	0.351	77.3 $\pm$ 22.5	ref.	80.8 $\pm$ 23.5	0.09	(-0.26;0.45)	0.613	0.915	0.245	0.318
Asparagine	Asn	43.5 $\pm$ 9.0	-0.02	(-0.16;0.13)	0.814	0.917	43.0 $\pm$ 9.0	ref.	44.2 $\pm$ 9.1	0.22	(-0.13;0.57)	0.214	0.915	0.743	0.718
Aspartate	Asp	11.8 $\pm$ 4.0	-0.14	(-0.28;0.01)	0.074	0.349	12.6 $\pm$ 4.3	ref.	11.1 $\pm$ 3.5	-0.28	(-0.64;0.07)	0.122	0.915	0.656	0.604
Citrulline	Cit	36.5 $\pm$ 10.2	0.04	(-0.11;0.19)	0.572	0.901	36.1 $\pm$ 10.5	ref.	35.2 $\pm$ 9.5	-0.10	(-0.46;0.26)	0.592	0.915	0.610	0.671

Glutamine	Gln	696.5 ± 98.5	-0.04	(-0.18;0.10)	0.618	0.901	689.9 ± 95.5	ref.	703.8 ± 95.9	0.08	(-0.26;0.42)	0.633	0.932	695.8 ± 104.9	0.12	(-0.23;0.47)	0.504	0.801	0.643	0.869
Glutamate	Glu	80.0 ± 29.7	0.00	(-0.14;0.15)	0.979	0.979	77.9 ± 26.5	ref.	78.5 ± 28.4	-0.05	(-0.40;0.30)	0.784	0.961	83.6 ± 33.7	-0.01	(-0.38;0.35)	0.947	0.993	0.793	0.876
Glycine	Gly	215.6 ± 59.8	-0.08	(-0.21;0.06)	0.249	0.610	229.8 ± 70.8	ref.	212.1 ± 50.6	-0.12	(-0.45;0.20)	0.464	0.915	204.8 ± 54	-0.14	(-0.48;0.20)	0.424	0.728	0.258	0.601
Histidine	His	71.1 ± 12.4	0.07	(-0.08;0.21)	0.362	0.766	69.4 ± 13.1	ref.	72.2 ± 10.4	0.14	(-0.21;0.49)	0.433	0.915	71.8 ± 13.5	0.10	(-0.26;0.46)	0.573	0.849	0.537	0.869
Isoleucine	Ile	75.4 ± 23.9	0.12	(-0.02;0.26)	0.086	0.351	67.7 ± 17.8	ref.	76.8 ± 25.4	0.23	(-0.10;0.57)	0.175	0.915	81.6 ± 26.0	0.45	(0.1;0.80)	0.012	0.095	0.010	0.104
Leucine	Leu	133.8 ± 42.3	0.14	(0.00;0.28)	0.054	0.303	121.4 ± 34.3	ref.	137 ± 41.4	0.27	(-0.07;0.61)	0.126	0.915	143 ± 47.8	0.36	(0.00;0.71)	0.050	0.223	0.047	0.234
Lysine	Lys	193.5 ± 41.8	0.13	(-0.01;0.27)	0.069	0.342	182.9 ± 33.9	ref.	196 ± 46.1	0.20	(-0.15;0.54)	0.264	0.915	201.6 ± 42.8	0.41	(0.05;0.76)	0.025	0.128	0.028	0.169
Methionine	Met	20.20 ± 5.8	0.13	(-0.01;0.28)	0.065	0.335	18.8 ± 5.0	ref.	20.3 ± 6.2	0.23	(-0.11;0.57)	0.185	0.915	21.6 ± 5.9	0.44	(0.09;0.79)	0.016	0.102	0.011	0.104
Ornithine	Orn	71.3 ± 24.6	0.03	(-0.12;0.17)	0.720	0.901	70.7 ± 28.9	ref.	70.1 ± 24.3	0.08	(-0.28;0.43)	0.677	0.961	73.1 ± 19.9	0.32	(-0.04;0.69)	0.084	0.311	0.087	0.341
Phenylalanine	Phe	62.8 ± 13.6	0.09	(-0.05;0.23)	0.226	0.584	59.7 ± 11.6	ref.	63.7 ± 14.6	0.16	(-0.18;0.51)	0.347	0.915	64.9 ± 14.2	0.24	(-0.11;0.60)	0.179	0.490	0.122	0.408
Proline	Pro	242.6 ± 79.5	0.04	(-0.10;0.18)	0.573	0.901	234.9 ± 74.4	ref.	249.9 ± 85.7	0.12	(-0.22;0.47)	0.486	0.915	243 ± 78.6	0.08	(-0.28;0.44)	0.664	0.888	0.737	0.876
Serine	Ser	93 ± 20.3	0.10	(-0.04;0.25)	0.169	0.513	90.1 ± 19.2	ref.	94.3 ± 21.4	0.26	(-0.10;0.61)	0.157	0.915	94.7 ± 20.3	0.31	(-0.06;0.67)	0.101	0.348	0.075	0.316
Threonine	Thr	108.7 ± 27.7	0.18	(0.04;0.33)	0.012	0.118	103.5 ± 26.0	ref.	109.9 ± 29.5	0.34	(-0.01;0.68)	0.057	0.915	112.9 ± 27.1	0.45	(0.09;0.80)	0.015	0.102	0.009	0.104
Tryptophan	Trp	53.1 ± 11.0	0.18	(0.04;0.32)	0.013	0.118	49.8 ± 11.1	ref.	54.1 ± 10.6	0.35	(0.01;0.70)	0.048	0.915	55.5 ± 10.6	0.49	(0.13;0.85)	0.008	0.081	0.009	0.104
Tyrosine	Tyr	74.8 ± 24.1	0.10	(-0.05;0.24)	0.195	0.555	67.5 ± 19.9	ref.	76.0 ± 22.8	0.30	(-0.05;0.65)	0.095	0.915	80.7 ± 27.4	0.45	(0.09;0.81)	0.016	0.102	0.012	0.104
Valine	Val	242.1 ± 64.0	0.17	(0.03;0.31)	0.019	0.141	221.0 ± 52.8	ref.	245.3 ± 59.5	0.26	(-0.08;0.60)	0.134	0.915	259.9 ± 73	0.43	(0.08;0.78)	0.018	0.106	0.018	0.126
BIOGENIC AMINES																				
Creatinine	Creatinin e	74.5 ± 17.4	0.05	(-0.06;0.17)	0.368	0.766	71.8 ± 16.2	ref.	73.3 ± 13.1	-0.10	(-0.38;0.19)	0.506	0.915	78.3 ± 21.3	0.00	(-0.29;0.30)	0.988	0.993	0.838	0.884
Kynurenine	Kynureni ne	2.7 ± 0.7	0.03	(-0.11;0.17)	0.697	0.901	2.6 ± 0.9	ref.	2.6 ± 0.6	-0.19	(-0.52;0.15)	0.276	0.915	2.8 ± 0.7	0.02	(-0.33;0.37)	0.904	0.974	0.798	0.876
Sarcosine	Sarcosine	4.4 ± 1.1	0.10	(-0.05;0.24)	0.185	0.540	4.4 ± 1.3	ref.	4.4 ± 1.1	0.16	(-0.19;0.52)	0.373	0.915	4.4 ± 0.9	0.17	(-0.20;0.54)	0.361	0.677	0.437	0.751
Serotonin	Serotonin	0.8 ± 0.6	-0.05	(-0.19;0.10)	0.546	0.901	0.8 ± 0.6		0.8 ± 0.6	-0.02	(-0.37;0.34)	0.934	0.988	0.7 ± 0.5	-0.10	(-0.46;0.27)	0.608	0.867	0.788	0.876
Taurine	Taurine	120.9 ± 49.4	-0.05	(-0.19;0.10)	0.545	0.901	125.2 ± 48.9		120.1 ± 50.7	-0.03	(-0.39;0.33)	0.875	0.984	117.2 ± 49	-0.05	(-0.43;0.32)	0.777	0.921	0.768	0.876

Trans 4-Hydroxyproline Asymmetric dimethylarginine Symmetric dimethylarginine	14-OH-Pro	10.2 ± 5.0	0.11	(-0.03;0.26)	0.116	0.408	9.6 ± 5.3	ref.	10.20 ± 5.5	0.06	(-0.29;0.40)	0.746	0.961	10.7 ± 4.2	0.20	(-0.16;0.56)	0.284	0.610	0.318	0.666
	ADMA	0.5 ± 0.1	-0.05	(-0.19;0.08)	0.418	0.788	0.5 ± 0.1	ref.	0.5 ± 0.1	-0.12	(-0.44;0.20)	0.459	0.915	0.5 ± 0.1	-0.03	(-0.36;0.30)	0.850	0.957	0.593	0.869
	SDMA	0.5 ± 0.1	-0.17	(-0.31;-0.04)	0.013	0.118	0.5 ± 0.1	ref.	0.5 ± 0.1	-0.16	(-0.49;0.17)	0.331	0.915	0.5 ± 0.1	-0.16	(-0.50;0.18)	0.358	0.677	0.251	0.601
GLYCEROPHOSPHOLIPIDS	LysoPC a C16:0	90.4 ± 20.1	0.08	(-0.07;0.23)	0.282	0.630	92.1 ± 21.2	ref.	90.7 ± 22.7	-0.05	(-0.42;0.31)	0.785	0.961	88.5 ± 15.9	-0.02	(-0.40;0.35)	0.909	0.974	0.654	0.869
	LysoPC a C16:1	2.6 ± 0.9	-0.02	(-0.17;0.13)	0.832	0.917	2.7 ± 1.1	ref.	2.5 ± 0.9	-0.17	(-0.53;0.19)	0.366	0.915	2.5 ± 0.8	-0.09	(-0.47;0.28)	0.636	0.888	0.437	0.751
	LysoPC a C17:0	1.6 ± 0.5	0.03	(-0.12;0.17)	0.730	0.901	1.7 ± 0.5	ref.	1.5 ± 0.5	-0.17	(-0.52;0.18)	0.334	0.915	1.5 ± 0.4	-0.07	(-0.43;0.29)	0.710	0.888	0.392	0.745
	LysoPC a C18:0	26.4 ± 6.8	0.10	(-0.04;0.25)	0.162	0.504	27.2 ± 7.5	ref.	26.4 ± 7.5	-0.02	(-0.38;0.33)	0.895	0.986	25.4 ± 5.2	0.01	(-0.36;0.38)	0.956	0.993	0.776	0.876
	LysoPC a C18:1	17.9 ± 5.4	-0.02	(-0.16;0.13)	0.823	0.917	18.8 ± 5.8	ref.	17.9 ± 5.9	-0.06	(-0.42;0.30)	0.740	0.961	17.1 ± 4.3	-0.07	(-0.43;0.30)	0.729	0.888	0.562	0.869
	LysoPC a C18:2	29.2 ± 10.4	-0.01	(-0.15;0.14)	0.932	0.952	29.8 ± 10.1	ref.	29.8 ± 10.9	-0.01	(-0.35;0.34)	0.966	0.988	28.1 ± 10.1	-0.04	(-0.40;0.31)	0.809	0.930	0.619	0.869
	LysoPC a C20:3	2.0 ± 0.7	-0.02	(-0.17;0.12)	0.770	0.901	2.2 ± 0.7	ref.	2.0 ± 0.8	-0.25	(-0.60;0.10)	0.158	0.915	1.9 ± 0.6	-0.25	(-0.61;0.11)	0.178	0.490	0.089	0.341
	LysoPC a C20:4	5.9 ± 1.7	0.12	(-0.02;0.27)	0.098	0.365	6.0 ± 2.0	ref.	5.7 ± 1.6	-0.08	(-0.44;0.27)	0.650	0.947	6.1 ± 1.6	0.18	(-0.19;0.55)	0.333	0.677	0.591	0.869
	LysoPC a C24:0	0.14 ± 0.03	0.11	(-0.02;0.24)	0.103	0.374	0.13 ± 0.04		0.13 ± 0.03	0.00	(-0.32;0.32)	0.988	0.988	0.14 ± 0.03	0.30	(-0.03;0.63)	0.079	0.306	0.172	0.490
	LysoPC a C28:1	0.3 ± 0.1	-0.05	(-0.17;0.07)	0.443	0.825	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.26	(-0.55;0.03)	0.084	0.915	0.3 ± 0.1	-0.20	(-0.49;0.10)	0.201	0.509	0.109	0.383
	PC aa C28:1	3.0 ± 0.8	-0.10	(-0.24;0.03)	0.143	0.491	3.1 ± 0.8	ref.	3.0 ± 0.7	-0.09	(-0.42;0.24)	0.584	0.915	2.9 ± 0.7	-0.09	(-0.43;0.25)	0.600	0.864	0.406	0.745
	PC aa C30:0	4.0 ± 1.3	-0.02	(-0.17;0.12)	0.766	0.901	4.1 ± 1.1	ref.	4.0 ± 1.3	-0.08	(-0.43;0.28)	0.679	0.961	4.0 ± 1.6	-0.03	(-0.40;0.34)	0.880	0.974	0.851	0.884
	PC aa C32:0	26.6 ± 5.5	0.02	(-0.13;0.16)	0.825	0.917	27 ± 4.9	ref.	26.0 ± 5.0	-0.10	(-0.45;0.24)	0.552	0.915	26.9 ± 6.5	0.09	(-0.27;0.44)	0.637	0.888	0.669	0.869
	PC aa C32:1	32.0 ± 16.2	-0.05	(-0.20;0.10)	0.508	0.884	33 ± 15.4	ref.	30.8 ± 14.0	-0.13	(-0.49;0.23)	0.467	0.915	32.3 ± 18.8	-0.08	(-0.45;0.29)	0.667	0.888	0.641	0.869
	PC aa C32:2	8.3 ± 3.1	-0.10	(-0.25;0.04)	0.161	0.504	8.7 ± 3.0	ref.	8.6 ± 2.9	-0.05	(-0.40;0.30)	0.779	0.961	7.8 ± 3.4	-0.20	(-0.56;0.17)	0.287	0.610	0.198	0.520
	PC aa C32:3	0.9 ± 0.2	-0.02	(-0.16;0.11)	0.718	0.901	0.9 ± 0.2	ref.	0.9 ± 0.2	0.04	(-0.28;0.37)	0.807	0.961	0.9 ± 0.3	-0.02	(-0.36;0.31)	0.897	0.974	0.681	0.869
	PC aa C34:1	36.5 ± 82.8	-0.05	(-0.20;0.09)	0.464	0.840	371.8 ± 86.9	ref.	364.3 ± 77.0	-0.04	(-0.40;0.31)	0.815	0.961	360.20 ± 85.1	-0.07	(-0.44;0.29)	0.693	0.888	0.706	0.872

PC (diacyl) C34:2	PC aa C34:2	739.8 ± 128.1	-0.06	-0.21;0.09	0.405	0.776	751.7 ± 130.8	ref.	746.8 ± 117.6	0.00	-0.36;0.36	0.983	0.988	720.8 ± 135	-0.15	-0.52;-0.23	0.441	0.743	0.423	0.751
PC (diacyl) C34:3	PC aa C34:3	30.1 ± 10.2	-0.04	-0.18;0.11	0.628	0.901	31.0 ± 10.9	ref.	31.0 ± 9.4	0.03	-0.32;0.37	0.882	0.984	28.4 ± 10.1	-0.15	-0.51;-0.21	0.421	0.728	0.397	0.745
PC (diacyl) C34:4	PC aa C34:4	3.1 ± 1.2	0.04	-0.10;0.18	0.584	0.901	3.0 ± 1.1	ref.	3.1 ± 1.1	0.04	-0.31;0.39	0.810	0.961	3.1 ± 1.3	0.17	-0.19;0.53	0.357	0.677	0.574	0.869
PC (diacyl) C36:0	PC aa C36:0	3.2 ± 1.0	0.22	0.09;0.36	0.002	0.029	3.1 ± 1.0	ref.	3.1 ± 0.9	0.18	-0.15;0.52	0.281	0.915	3.5 ± 1.2	0.59	0.25;0.94	0.001	0.025	0.003	0.077
PC (diacyl) C36:1	PC aa C36:1	62.9 ± 17.8	-0.01	-0.14;0.13	0.938	0.952	64.3 ± 18.3	ref.	62.3 ± 15.8	-0.01	-0.34;0.33	0.975	0.988	62.2 ± 19.2	0.06	-0.29;0.41	0.726	0.888	0.749	0.876
PC (diacyl) C36:2	PC aa C36:2	384.7 ± 79.7	-0.02	-0.16;0.12	0.767	0.901	393.9 ± 81.8	ref.	387.1 ± 72.4	-0.02	-0.36;0.32	0.917	0.988	373.3 ± 84.2	-0.07	-0.42;0.28	0.701	0.888	0.631	0.869
PC (diacyl) C36:3	PC aa C36:3	192.6 ± 43.8	-0.06	-0.20;0.08	0.388	0.766	200.6 ± 42.7	ref.	192.9 ± 42.7	-0.18	-0.51;0.16	0.311	0.915	184.4 ± 45.7	-0.23	-0.58;0.12	0.193	0.507	0.135	0.430
PC (diacyl) C36:4	PC aa C36:4	291.5 ± 72.0	0.14	0.00;0.28	0.054	0.303	282.8 ± 72.9	ref.	285.8 ± 65.8	0.05	-0.29;0.39	0.762	0.961	305.8 ± 75.9	0.37	0.02;0.72	0.042	0.208	0.098	0.355
PC (diacyl) C36:5	PC aa C36:5	45.8 ± 27.8	0.09	-0.05;0.24	0.217	0.584	44.1 ± 25.5	ref.	44.3 ± 24.5	0.07	-0.29;0.42	0.713	0.961	48.8 ± 32.8	0.26	-0.10;0.63	0.162	0.476	0.181	0.495
PC (diacyl) C36:6	PC aa C36:6	1.5 ± 0.6	0.12	-0.02;0.26	0.097	0.365	1.5 ± 0.6	ref.	1.5 ± 0.7	0.10	-0.24;0.45	0.561	0.915	1.6 ± 0.6	0.29	-0.07;0.65	0.114	0.379	0.223	0.554
PC (diacyl) C38:0	PC aa C38:0	2.9 ± 0.9	0.32	0.18;0.45	0.000	0.001	2.7 ± 0.9	ref.	2.8 ± 0.8	0.31	-0.01;0.64	0.060	0.915	3.1 ± 0.9	0.67	0.33;1.01	0.0001	0.009	0.0002	0.020
PC (diacyl) C38:3	PC aa C38:3	53.2 ± 13.6	0.04	-0.09;0.18	0.525	0.901	54.4 ± 11.7	ref.	52.3 ± 13	-0.19	-0.51;0.13	0.248	0.915	53.0 ± 15.9	-0.02	-0.36;0.31	0.893	0.974	0.676	0.869
PC (diacyl) C38:4	PC aa C38:4	131.5 ± 35.0	0.19	0.06;0.32	0.004	0.054	127.8 ± 34.4	ref.	126.6 ± 28.5	0.01	-0.30;0.33	0.931	0.988	140.20 ± 40	0.42	0.1;0.75	0.012	0.095	0.036	0.185
PC (diacyl) C38:5	PC aa C38:5	66.2 ± 19.0	0.09	-0.04;0.23	0.184	0.540	66.3 ± 17.8	ref.	64.8 ± 18.2	-0.01	-0.34;0.32	0.951	0.988	67.6 ± 21.1	0.22	-0.12;0.57	0.210	0.521	0.302	0.643
PC (diacyl) C38:6	PC aa C38:6	101.1 ± 33.6	0.27	0.13;0.40	0.000	0.006	95.3 ± 31.1	ref.	100.1 ± 35.2	0.19	-0.15;0.53	0.283	0.915	107.9 ± 33.6	0.51	0.16;0.86	0.005	0.062	0.012	0.104
PC (diacyl) C40:2	PC aa C40:2	0.3 ± 0.1	0.01	-0.13;0.15	0.855	0.924	0.3 ± 0.1	ref.	0.3 ± 0.1	0.05	-0.28;0.39	0.753	0.961	0.3 ± 0.1	0.25	-0.10;0.60	0.164	0.476	0.175	0.490
PC (diacyl) C40:3	PC aa C40:3	0.4 ± 0.1	0.01	-0.13;0.15	0.909	0.944	0.4 ± 0.1	ref.	0.4 ± 0.1	-0.11	-0.44;0.22	0.515	0.915	0.4 ± 0.2	0.19	-0.15;0.53	0.275	0.610	0.349	0.697
PC (diacyl) C40:4	PC aa C40:4	3.0 ± 0.8	0.08	-0.06;0.23	0.274	0.623	3.0 ± 0.8	ref.	2.9 ± 0.7	-0.04	-0.39;0.31	0.834	0.961	3.1 ± 1	0.16	-0.20;0.53	0.375	0.688	0.508	0.840
PC (diacyl) C40:5	PC aa C40:5	8.3 ± 2.4	0.06	-0.08;0.20	0.394	0.766	8.2 ± 2.0	ref.	8.2 ± 2.3	-0.03	-0.38;0.31	0.844	0.961	8.4 ± 3	0.07	-0.29;0.43	0.710	0.888	0.774	0.876
PC (diacyl) C40:6	PC aa C40:6	28.3 ± 10.2	0.29	0.16;0.43	0.000	0.002	26.8 ± 9.1	ref.	27.3 ± 10.2	0.10	-0.23;0.43	0.565	0.915	30.8 ± 10.9	0.50	0.16;0.84	0.005	0.062	0.010	0.104
PC (diacyl) C42:0	PC aa C42:0	0.3 ± 0.1	0.21	0.08;0.34	0.002	0.035	0.4 ± 0.1	ref.	0.3 ± 0.1	0.17	-0.15;0.50	0.299	0.915	0.3 ± 0.1	0.34	0.00;0.68	0.050	0.223	0.068	0.316
PC (diacyl) C42:1	PC aa C42:1	0.18 ± 0.05	0.23	0.1;0.36	0.001	0.016	0.2 ± 0.1	ref.	0.17 ± 0.04	0.12	-0.20;0.45	0.455	0.915	0.2 ± 0.1	0.45	0.12;0.78	0.009	0.088	0.018	0.126

PC (diacyl) C42:2	PC aa C42:2	0.16 ± 0.04	0.13	-0.01;0.27	0.076	0.349	0.15 ± 0.04	ref.	0.16 ± 0.04	0.22	-0.11;-0.56	0.190	0.915	0.16 ± 0.05	0.50	(0.15;0.84)	0.006	0.062	0.010	0.104
PC (diacyl) C42:4	PC aa C42:4	0.10 ± 0.02	0.04	(-0.11;0.18)	0.620	0.901	0.10 ± 0.02	ref.	0.10 ± 0.02	0.04	(-0.32;0.39)	0.847	0.961	0.11 ± 0.02	0.24	(-0.12;-0.61)	0.194	0.507	0.260	0.601
PC (diacyl) C42:5	PC aa C42:5	0.3 ± 0.1	0.03	(-0.11;0.18)	0.658	0.901	0.3 ± 0.1	ref.	0.2 ± 0.1	-0.12	(-0.48;0.23)	0.489	0.915	0.3 ± 0.1	0.17	(-0.20;0.53)	0.364	0.677	0.404	0.745
PC (diacyl) C42:6	PC aa C42:6	0.4 ± 0.1	0.02	(-0.12;0.17)	0.737	0.901	0.4 ± 0.1	ref.	0.4 ± 0.1	-0.02	(-0.37;0.33)	0.916	0.988	0.4 ± 0.2	0.21	(-0.15;-0.58)	0.244	0.564	0.283	0.622
PC (acyl-alkyl) C30:0	PC ae C30:0	0.3 ± 0.1	-0.06	(-0.20;0.08)	0.391	0.766	0.4 ± 0.1	ref.	0.3 ± 0.1	-0.15	(-0.49;0.19)	0.375	0.915	0.3 ± 0.1	-0.20	(-0.55;0.16)	0.276	0.610	0.209	0.527
PC (acyl-alkyl) C30:2	PC ae C30:2	0.08 ± 0.02	0.01	(-0.11;0.14)	0.835	0.917	0.09 ± 0.02	ref.	0.08 ± 0.02	-0.10	(-0.40;0.20)	0.519	0.915	0.08 ± 0.02	-0.04	(-0.35;-0.27)	0.812	0.930	0.541	0.869
PC (acyl-alkyl) C32:1	PC ae C32:1	5.3 ± 1.2	-0.03	(-0.17;0.11)	0.657	0.901	5.4 ± 1.2	ref.	5.1 ± 1	-0.10	(-0.44;0.24)	0.556	0.915	5.3 ± 1.4	0.08	(-0.27;0.43)	0.668	0.888	0.812	0.877
PC (acyl-alkyl) C32:2	PC ae C32:2	1.4 ± 0.4	-0.01	(-0.15;0.12)	0.851	0.924	1.4 ± 0.4	ref.	1.3 ± 0.3	-0.11	(-0.43;0.22)	0.511	0.915	1.4 ± 0.4	0.20	(-0.13;-0.54)	0.241	0.564	0.400	0.745
PC (acyl-alkyl) C34:0	PC ae C34:0	2.5 ± 0.6	0.00	(-0.14;0.14)	0.976	0.979	2.5 ± 0.6	ref.	2.4 ± 0.6	-0.09	(-0.43;0.25)	0.601	0.915	2.4 ± 0.7	0.00	(-0.35;0.36)	0.990	0.993	0.939	0.960
PC (acyl-alkyl) C34:1	PC ae C34:1	18.0 ± 3.9	-0.10	(-0.23;0.03)	0.147	0.491	19.1 ± 4.2	ref.	17.7 ± 3.3	-0.12	(-0.45;0.20)	0.458	0.915	17.3 ± 3.9	-0.16	(-0.50;0.18)	0.361	0.677	0.341	0.693
PC (acyl-alkyl) C34:2	PC ae C34:2	20.2 ± 5.3	0.03	(-0.11;0.17)	0.690	0.901	20.4 ± 5.5	ref.	20.9 ± 5.1	0.19	(-0.15;0.52)	0.275	0.915	19.4 ± 5.3	0.05	(-0.30;0.39)	0.797	0.930	0.811	0.877
PC (acyl-alkyl) C34:3	PC ae C34:3	13.1 ± 4.0	0.03	(-0.11;0.17)	0.679	0.901	13.2 ± 4.3	ref.	13.6 ± 3.8	0.18	(-0.16;0.51)	0.310	0.915	12.6 ± 3.8	0.06	(-0.28;0.41)	0.720	0.888	0.797	0.876
PC (acyl-alkyl) C36:0	PC ae C36:0	1.2 ± 0.3	0.06	(-0.09;0.20)	0.452	0.830	1.1 ± 0.3	ref.	1.2 ± 0.3	0.12	(-0.24;0.47)	0.522	0.915	1.2 ± 0.4	0.23	(-0.13;-0.60)	0.218	0.531	0.149	0.465
PC (acyl-alkyl) C36:1	PC ae C36:1	11.5 ± 2.7	-0.08	(-0.21;0.06)	0.250	0.610	12.2 ± 3.2	ref.	11.2 ± 2.2	-0.13	(-0.45;0.20)	0.455	0.915	11 ± 2.4	-0.10	(-0.44;0.24)	0.576	0.849	0.437	0.751
PC (acyl-alkyl) C36:2	PC ae C36:2	21.7 ± 5.4	-0.07	(-0.21;0.06)	0.272	0.623	23.3 ± 6.1	ref.	21.5 ± 4.8	-0.12	(-0.44;0.20)	0.472	0.915	20.3 ± 4.8	-0.20	(-0.53;-0.13)	0.239	0.564	0.156	0.473
PC (acyl-alkyl) C36:3	PC ae C36:3	11.4 ± 2.8	0.03	(-0.10;0.17)	0.641	0.901	11.6 ± 3.0	ref.	11.7 ± 2.7	0.14	(-0.19;0.47)	0.404	0.915	10.9 ± 2.8	0.01	(-0.33;0.35)	0.950	0.993	0.982	0.990
PC (acyl-alkyl) C36:4	PC ae C36:4	26.3 ± 6.4	0.24	(0.1;0.38)	0.001	0.018	24.8 ± 6.0	ref.	26.4 ± 6.2	0.25	(-0.10;0.59)	0.159	0.915	27.5 ± 6.7	0.52	(0.16;0.87)	0.005	0.062	0.012	0.104
PC (acyl-alkyl) C36:5	PC ae C36:5	18.3 ± 5.0	0.18	(0.04;0.32)	0.011	0.118	17.2 ± 4.3	ref.	17.9 ± 4.5	0.19	(-0.14;0.51)	0.263	0.915	19.8 ± 5.7	0.66	(0.32;0.99)	0.0002	0.009	0.001	0.052
PC (acyl-alkyl) C38:0	PC ae C38:0	2.5 ± 0.8	0.16	(0.02;0.29)	0.029	0.177	2.4 ± 0.7	ref.	2.5 ± 0.8	0.22	(-0.11;0.56)	0.193	0.915	2.6 ± 0.9	0.44	(0.1;-0.79)	0.013	0.095	0.031	0.174
PC (acyl-alkyl) C38:2	PC ae C38:2	2.2 ± 0.6	-0.03	(-0.17;0.10)	0.634	0.901	2.3 ± 0.6	ref.	2.1 ± 0.5	-0.16	(-0.49;0.17)	0.350	0.915	2.1 ± 0.5	-0.04	(-0.39;0.30)	0.804	0.930	0.569	0.869
PC (acyl-alkyl) C38:3	PC ae C38:3	4.8 ± 1.1	-0.04	(-0.17;0.10)	0.603	0.901	5.1 ± 1.2	ref.	4.6 ± 1.0	-0.32	(-0.64;-0.01)	0.046	0.915	4.5 ± 1	-0.24	(-0.57;0.09)	0.154	0.468	0.076	0.316
PC (acyl-alkyl) C38:4	PC ae C38:4	17.2 ± 3.6	0.16	(0.03;0.30)	0.020	0.141	17.2 ± 4.0	ref.	17.0 ± 3.2	0.10	(-0.23;0.44)	0.550	0.915	17.5 ± 3.7	0.33	(-0.02;-0.67)	0.064	0.267	0.160	0.473

PC (acyl-alkyl) C38:5	PC ae C38:5	21.9 ± 4.7	0.20	(0.06;0.34)	0.004	0.054	21.2 ± 4.6	ref.	21.8 ± 4.3	0.24	(-0.09;0.57)	0.149	0.915	22.9 ± 4.9	0.58	(0.24;0.92)	0.001	0.025	0.003	0.077
PC (acyl-alkyl) C38:6	PC ae C38:6	9.2 ± 2.6	0.26	(0.12;0.39)	0.000	0.006	8.7 ± 2.4	ref.	9.1 ± 2.5	0.24	(-0.08;0.56)	0.144	0.915	9.9 ± 2.8	0.68	(0.35;1.01)	0.0001	0.009	0.0003	0.020
PC (acyl-alkyl) C40:1	PC ae C40:1	1.1 ± 0.3	0.17	(0.03;0.31)	0.018	0.141	1.1 ± 0.3	ref.	1.1 ± 0.3	0.09	(-0.25;0.43)	0.614	0.915	1.2 ± 0.3	0.51	(0.17;0.86)	0.004	0.062	0.015	0.120
PC (acyl-alkyl) C40:2	PC ae C40:2	1.6 ± 0.4	-0.02	(-0.15;0.12)	0.819	0.917	1.6 ± 0.4	ref.	1.6 ± 0.4	0.09	(-0.24;0.42)	0.595	0.915	1.6 ± 0.5	0.15	(-0.20;0.49)	0.401	0.716	0.466	0.781
PC (acyl-alkyl) C40:3	PC ae C40:3	0.8 ± 0.2	-0.01	(-0.14;0.12)	0.880	0.936	0.9 ± 0.2	ref.	0.8 ± 0.2	-0.06	(-0.37;0.26)	0.712	0.961	0.8 ± 0.2	-0.03	(-0.36;0.29)	0.848	0.957	0.662	0.869
PC (acyl-alkyl) C40:4	PC ae C40:4	2.2 ± 0.4	0.13	(-0.01;0.27)	0.064	0.335	2.2 ± 0.5	ref.	2.2 ± 0.4	0.17	(-0.16;0.50)	0.314	0.915	2.2 ± 0.5	0.27	(-0.07;0.62)	0.120	0.383	0.162	0.473
PC (acyl-alkyl) C40:5	PC ae C40:5	3.4 ± 0.7	0.12	(-0.02;0.25)	0.090	0.353	3.4 ± 0.7	ref.	3.3 ± 0.6	0.14	(-0.19;0.46)	0.420	0.915	3.4 ± 0.8	0.26	(-0.08;0.60)	0.139	0.433	0.189	0.508
PC (acyl-alkyl) C40:6	PC ae C40:6	4.8 ± 1.2	0.26	(0.12;0.39)	0.000	0.006	4.7 ± 1.3	ref.	4.7 ± 1.1	0.19	(-0.13;0.51)	0.253	0.915	4.9 ± 1.2	0.51	(0.18;0.85)	0.003	0.062	0.009	0.104
PC (acyl-alkyl) C42:1	PC ae C42:1	0.3 ± 0.1	0.12	(-0.01;0.26)	0.082	0.351	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.05	(-0.38;0.28)	0.756	0.961	0.3 ± 0.1	0.27	(-0.07;0.62)	0.116	0.379	0.289	0.625
PC (acyl-alkyl) C42:2	PC ae C42:2	0.4 ± 0.1	0.06	(-0.07;0.19)	0.391	0.766	0.4 ± 0.1	ref.	0.4 ± 0.1	0.16	(-0.16;0.49)	0.322	0.915	0.4 ± 0.1	0.24	(-0.10;0.57)	0.167	0.477	0.202	0.520
PC (acyl-alkyl) C42:3	PC ae C42:3	0.6 ± 0.1	0.08	(-0.06;0.22)	⁵ 0.256	0.614	0.6 ± 0.1	ref.	0.6 ± 0.1	0.14	(-0.19;0.47)	¹ 0.414	0.915	0.5 ± 0.1	0.23	(-0.12;0.57)	0.1969	0.507	0.270	0.604
PC (acyl-alkyl) C42:4	PC ae C42:4	0.7 ± 0.2	0.05	(-0.09;0.19)	0.500	0.881	0.7 ± 0.2	ref.	0.7 ± 0.1	0.15	(-0.19;0.48)	0.388	0.915	0.7 ± 0.2	0.05	(-0.29;0.40)	0.763	0.920	0.786	0.876
PC (acyl-alkyl) C42:5	PC ae C42:5	1.5 ± 0.3	0.16	(0.03;0.30)	0.020	0.141	1.5 ± 0.3	ref.	1.5 ± 0.3	0.22	(-0.11;0.56)	0.197	0.915	1.5 ± 0.4	0.36	(0.01;0.70)	0.046	0.221	0.072	0.316
PC (acyl-alkyl) C44:3	PC ae C44:3	0.07 ± 0.02	0.08	(-0.06;0.21)	0.266	0.623	0.07 ± 0.02	ref.	0.07 ± 0.02	0.10	(-0.23;0.42)	0.555	0.915	0.07 ± 0.02	0.41	(0.07;0.74)	0.018	0.106	0.027	0.169
PC (acyl-alkyl) C44:4	PC ae C44:4	0.2 ± 0.1	0.01	(-0.13;0.15)	0.920	0.948	0.2 ± 0.1	ref.	0.2 ± 0.1	0.01	(-0.33;0.35)	0.971	0.988	0.2 ± 0.1	-0.01	(-0.36;0.35)	0.978	0.993	0.882	0.909
PC (acyl-alkyl) C44:5	PC ae C44:5	1.0 ± 0.3	0.13	(-0.02;0.27)	0.082	0.351	1.0 ± 0.3	ref.	1.0 ± 0.2	0.30	(-0.05;0.64)	0.094	0.915	1.0 ± 0.3	0.31	(-0.05;0.67)	0.089	0.322	0.092	0.343
PC (acyl-alkyl) C44:6	PC ae C44:6	0.8 ± 0.2	0.15	(0.02;0.29)	0.026	0.170	0.8 ± 0.2	ref.	0.8 ± 0.2	0.17	(-0.16;0.50)	0.318	0.915	0.8 ± 0.3	0.32	(-0.03;0.66)	0.072	0.293	0.113	0.390
SPHINGOLIPIDS																				
Hydroxysphingomyelin C14:1	SM OH C14:1	4.0 ± 1.0	-0.09	(-0.21;0.04)	0.162	0.504	4.2 ± 1.2	ref.	4.0 ± 0.9	-0.04	(-0.34;0.27)	0.819	0.961	3.8 ± 1.0	-0.06	(-0.37;0.26)	0.710	0.888	0.596	0.869
Hydroxysphingomyelin C16:1	SM OH C16:1	1.9 ± 0.5	-0.06	(-0.19;0.07)	0.338	0.731	1.9 ± 0.5	ref.	1.8 ± 0.4	0.02	(-0.30;0.34)	0.898	0.986	1.8 ± 0.5	0.01	(-0.31;0.34)	0.932	0.991	0.998	0.998
Hydroxysphingomyelin C22:1	SM OH C22:1	4.4 ± 1.1	-0.02	(-0.15;0.11)	0.768	0.901	4.5 ± 1.1	ref.	4.5 ± 1.0	0.17	(-0.15;0.49)	0.295	0.915	4.2 ± 1.0	0.07	(-0.26;0.40)	0.675	0.888	0.794	0.876

Hydroxysphingomyelin C22:2	SM OH	-0.02	(-0.14;0.11)	0.771	0.901	3.4 ± 1.0	ref.	3.3 ± 0.8	0.15	(-0.15;0.45)	0.330	0.915	3.2 ± 0.9	0.12	(-0.19;0.43)	0.448	0.743	0.606	0.869
Hydroxysphingomyelin C24:1	SM OH	0.02	(-0.12;0.16)	0.773	0.901	0.3 ± 0.1	ref.	0.3 ± 0.1	0.17	(-0.17;0.51)	0.329	0.915	0.3 ± 0.1	0.11	(-0.24;-0.47)	0.527	0.812	0.680	0.869
Sphingomyelin C16:0	SM	-0.02	(-0.15;0.11)	0.714	0.901	68.1 ± 12.4	ref.	67.7 ± 12.3	0.12	(-0.20;0.43)	0.471	0.915	65.8 ± 15.9	0.10	(-0.22;0.43)	0.533	0.812	0.663	0.869
Sphingomyelin C16:1	SM	0.03	(-0.09;0.16)	0.605	0.901	9.5 ± 2.2	ref.	9.6 ± 2.1	0.09	(-0.21;0.39)	0.547	0.915	9.3 ± 2.3	0.13	(-0.18;-0.44)	0.410	0.722	0.709	0.872
Sphingomyelin C18:0	SM	-0.03	(-0.17;0.10)	0.638	0.901	12.1 ± 3.4	ref.	12.0 ± 2.6	0.10	(-0.23;0.43)	0.538	0.915	12 ± 3.8	0.09	(-0.25;0.44)	0.589	0.858	0.619	0.869
Sphingomyelin C18:1	SM	0.03	(-0.10;0.16)	0.662	0.901	5.6 ± 1.7	ref.	5.7 ± 1.4	0.15	(-0.16;0.46)	0.349	0.915	5.7 ± 1.8	0.17	(-0.15;0.49)	0.299	0.626	0.415	0.751
Sphingomyelin C20:2	SM	-0.08	(-0.21;0.05)	0.218	0.584	0.2 ± 0.1	ref.	0.14 ± 0.05	-0.19	(-0.50;0.13)	0.249	0.915	0.1 ± 0.1	-0.16	(-0.48;0.17)	0.351	0.677	0.258	0.601
Sphingomyelin C24:0	SM	0.03	(-0.11;0.17)	0.652	0.901	6.1 ± 1.4	ref.	6.4 ± 1.4	0.25	(-0.08;0.59)	0.140	0.915	5.9 ± 1.4	0.12	(-0.22;0.47)	0.491	0.801	0.614	0.869
Sphingomyelin C24:1	SM	0.04	(-0.10;0.18)	0.566	0.901	13.8 ± 3.2	ref.	13.9 ± 2.8	0.24	(-0.09;0.57)	0.163	0.915	13.9 ± 4.2	0.29	(-0.05;0.64)	0.097	0.340	0.132	0.430
Sphingomyelin C26:0	SM	-0.05	(-0.19;0.09)	0.495	0.881	0.04 ± 0.01	ref.	0.04 ± 0.01	0.20	(-0.15;0.54)	0.272	0.915	0.03 ± 0.01	0.00	(-0.36;0.36)	0.993	0.993	0.822	0.879
Sphingomyelin C26:1	SM	0.08	(-0.07;0.22)	0.300	0.660	0.10 ± 0.03	ref.	0.10 ± 0.03	0.09	(-0.25;0.44)	0.603	0.915	0.1 ± 0.1	0.46	(0.1;-0.82)	0.013	0.095	0.029	0.169
HEXOSES																			
Hexoses	H1	-0.01	(-0.16;0.13)	0.868	0.931	5496.6 ± 1445.1	ref.	5583.0 ± 1605.3	-0.06	(-0.42;0.29)	0.721	0.961	5642.3 ± 1405.3	0.00	(-0.36;0.37)	0.979	0.993	0.951	0.965

Prudent pattern

Metabolite	Abbrevi ation	Mean $\pm$ SD ²	Continuous			Tertile 1		Tertile 2		Tertile 3				p_{trend}	p_{FDR}^4	
			β	95% CI ³	p - value	Mean $\pm$ SD ²	β	Mean $\pm$ SD ²	β	95% CI ³	p - value	Mean $\pm$ SD ²	β			95% CI ³
ACYLCARNITINES																
Carnitine	C0	36.0 $\pm$ 7.3	-0.05	(-0.22;-0.13)	0.606	0.882	37.3 $\pm$ 7.8	ref.	35.4 $\pm$ 7.3	-0.13	(-0.49;0.23)	0.476	0.907	0.875	0.712	0.868
Acetylcarnitine	C2	6.0 $\pm$ 1.9	-0.10	(-0.27;-0.07)	0.250	0.598	6.2 $\pm$ 2	ref.	6.0 $\pm$ 2.2	-0.10	(-0.45;0.26)	0.600	0.907	0.686	0.338	0.604
Propionylcarnitine	C3	0.5 $\pm$ 0.2	0.01	(-0.16;-0.18)	0.905	0.947	0.5 $\pm$ 0.2	ref.	0.5 $\pm$ 0.1	0.13	(-0.22;0.49)	0.467	0.907	0.786	0.497	0.754
Butyrylcarnitine	C4	0.2 $\pm$ 0.1	0.04	(-0.14;-0.21)	0.678	0.908	0.2 $\pm$ 0.1	ref.	0.2 $\pm$ 0.1	0.06	(-0.30;0.42)	0.747	0.907	0.849	0.664	0.845
Valeryl carnitine	C5	0.2 $\pm$ 0.1	0.02	(-0.14;-0.18)	0.811	0.947	0.2 $\pm$ 0.1	ref.	0.2 $\pm$ 0.1	0.08	(-0.26;0.41)	0.663	0.907	0.759	0.529	0.775
Decenoylcarnitine	C10:1	0.1 $\pm$ 0.1	0.01	(-0.16;-0.18)	0.875	0.947	0.1 $\pm$ 0.1	ref.	0.1 $\pm$ 0.1	0.30	(-0.05;0.66)	0.097	0.728	0.880	0.936	0.960
Tetradecenoylcarnitine	C14:1	0.05 $\pm$ 0.02	-0.12	(-0.28;-0.05)	0.178	0.523	0.06 $\pm$ 0.03	ref.	0.06 $\pm$ 0.03	-0.06	(-0.41;0.30)	0.748	0.907	0.522	0.255	0.542
Tetradecadienylcarnitin e	C14:2	0.02 $\pm$ 0.01	-0.11	(-0.27;-0.06)	0.214	0.531	0.03 $\pm$ 0.01	ref.	0.02 $\pm$ 0.01	0.03	(-0.32;0.38)	0.875	0.938	0.537	0.274	0.562
Hexadecanoylcarnitine	C16	0.10 $\pm$ 0.03	-0.27	(-0.44;-0.11)	0.001	0.056	0.11 $\pm$ 0.03	ref.	0.10 $\pm$ 0.03	-0.39	(-0.74;-0.05)	0.027	0.685	0.003	0.003	0.098
Octadecanoylcarnitine	C18	0.05 $\pm$ 0.01	-0.23	(-0.40;-0.07)	0.006	0.098	0.05 $\pm$ 0.01	ref.	0.05 $\pm$ 0.01	-0.32	(-0.67;0.03)	0.079	0.728	0.012	0.168	0.188
Octadecenoylcarnitine	C18:1	0.09 $\pm$ 0.03	-0.28	(-0.45;-0.11)	0.001	0.056	0.09 $\pm$ 0.03	ref.	0.09 $\pm$ 0.03	-0.21	(-0.57;0.15)	0.262	0.907	0.007	0.111	0.131
Octadecadienylcarnitin e	C18:2	0.03 $\pm$ 0.01	-0.28	(-0.45;-0.11)	0.002	0.056	0.04 $\pm$ 0.02	ref.	0.04 $\pm$ 0.01	-0.05	(-0.41;0.31)	0.790	0.907	0.005	0.091	0.118
AMINO ACIDS																
Alanine	Ala	382.8 $\pm$ 100.7	-0.14	(-0.31;-0.03)	0.117	0.471	389.8 $\pm$ 99.7	ref.	396.1 $\pm$ 112.8	0.02	(-0.35;0.38)	0.929	0.944	0.480	0.242	0.542
Arginine	Arg	81.3 $\pm$ 23.5	0.15	(-0.02;0.32)	0.078	0.388	79.2 $\pm$ 24.2	ref.	79.1 $\pm$ 20.2	0.16	(-0.20;0.52)	0.385	0.907	0.315	0.070	0.350
Asparagine	Asn	43.5 $\pm$ 9.0	0.04	(-0.13;0.20)	0.675	0.908	43.0 $\pm$ 8.6	ref.	43.8 $\pm$ 9.2	0.06	(-0.30;0.41)	0.752	0.907	0.968	0.911	0.961
Aspartate	Asp	11.8 $\pm$ 4.0	-0.12	(-0.30;0.05)	0.167	0.517	12.1 $\pm$ 3.5	ref.	12.3 $\pm$ 4.5	-0.06	(-0.42;0.30)	0.727	0.907	0.017	0.195	0.199
Citrulline	Cit	36.5 $\pm$ 10.2	-0.08	(-0.25;-0.10)	0.387	0.720	36.2 $\pm$ 8.9	ref.	39.3 $\pm$ 10.1	0.22	(-0.13;0.58)	0.221	0.907	0.327	0.118	0.369

Glutamine	696.5 ± 98.5	-0.06	(-0.22;0.11)	0.499	0.787	699.5 ± 108	ref.	697 ± 99	0.10	(-0.24;0.45)	0.565	0.907	693.1 ± 89	-0.03	(-0.39;0.33)	0.857	0.936	0.816	0.912
Glutamate	80.0 ± 29.7	0.00	(-0.17;0.17)	0.980	0.983	85.8 ± 30.9	ref.	78.5 ± 29.7	-0.21	(-0.56;0.15)	0.260	0.907	75.6 ± 27.8	-0.27	(-0.64;0.10)	0.161	0.431	0.153	0.433
Glycine	215.6 ± 59.8	-0.21	(-0.36;-0.05)	0.010	0.113	218.6 ± 64	ref.	215.8 ± 63.9	-0.09	(-0.42;0.24)	0.584	0.907	212.4 ± 51.3	-0.30	(-0.65;0.04)	0.085	0.327	0.062	0.329
Histidine	71.1 ± 12.4	0.03	(-0.13;0.20)	0.696	0.919	71.6 ± 11.9	ref.	69.9 ± 12.3	-0.02	(-0.37;0.33)	0.917	0.944	71.8 ± 13.2	0.12	(-0.24;-0.49)	0.514	0.774	0.500	0.754
Isoleucine	75.4 ± 23.9	0.08	(-0.09;0.24)	0.364	0.707	75.4 ± 18.7	ref.	73.6 ± 27.4	-0.06	(-0.41;0.28)	0.713	0.907	77.1 ± 25.2	0.22	(-0.14;0.58)	0.229	0.511	0.253	0.542
Leucine	133.8 ± 42.3	0.13	(-0.04;0.30)	0.125	0.471	133.3 ± 33.7	ref.	129.5 ± 46	-0.02	(-0.37;0.32)	0.894	0.944	138.6 ± 46.2	0.31	(-0.06;0.67)	0.100	0.338	0.116	0.369
Lysine	193.5 ± 41.8	0.17	(0.00;0.33)	0.050	0.320	191.8 ± 37.6	ref.	185.2 ± 38.9	-0.05	(-0.39;0.30)	0.788	0.907	203.4 ± 46.8	0.38	(0.02;0.74)	0.039	0.263	0.051	0.329
Methionine	20.20 ± 5.8	0.11	(-0.06;0.28)	0.195	0.528	19.8 ± 5.7	ref.	19.9 ± 5.4	0.00	(-0.35;0.35)	0.998	0.998	21.0 ± 6.3	0.34	(-0.02;0.70)	0.068	0.327	0.058	0.329
Ornithine	71.3 ± 24.6	-0.14	(-0.31;0.03)	0.111	0.471	74.7 ± 26.8	ref.	71.6 ± 24.4	-0.14	(-0.49;0.22)	0.452	0.907	67.6 ± 22.2	-0.38	(-0.75;-0.01)	0.045	0.263	0.050	0.329
Phenylalanine	62.8 ± 13.6	-0.03	(-0.20;0.13)	0.718	0.934	63.1 ± 11.7	ref.	62.1 ± 14.3	-0.07	(-0.41;0.28)	0.711	0.907	63.1 ± 14.8	0.03	(-0.33;0.39)	0.863	0.936	0.815	0.912
Proline	242.6 ± 79.5	0.06	(-0.11;0.22)	0.495	0.787	238.3 ± 76.6	ref.	243.8 ± 85.3	0.09	(-0.26;0.45)	0.602	0.907	245.7 ± 77.5	0.18	(-0.19;0.54)	0.349	0.623	0.398	0.683
Serine	93 ± 20.3	-0.06	(-0.24;0.11)	0.461	0.774	94.0 ± 20.3	ref.	90.5 ± 22.3	-0.27	(-0.63;0.09)	0.146	0.781	94.6 ± 18.2	-0.04	(-0.41;0.34)	0.841	0.936	0.835	0.922
Threonine	108.7 ± 27.7	0.00	(-0.16;0.17)	0.960	0.982	108.0 ± 29.7	ref.	111.3 ± 28.5	0.06	(-0.29;0.42)	0.734	0.907	106.9 ± 25	0.06	(-0.31;-0.43)	0.745	0.875	0.659	0.845
Tryptophan	53.1 ± 11.0	0.18	(0.01;0.35)	0.036	0.252	52.5 ± 12.2	ref.	52.1 ± 10.0	0.13	(-0.23;0.48)	0.482	0.907	54.8 ± 10.6	0.44	(0.08;0.81)	0.019	0.195	0.019	0.199
Tyrosine	74.8 ± 24.1	0.12	(-0.05;0.29)	0.180	0.523	71.9 ± 21.3	ref.	74.7 ± 26.0	0.07	(-0.28;0.43)	0.686	0.907	77.6 ± 24.8	0.32	(-0.05;0.69)	0.093	0.338	0.085	0.369
Valine	242.1 ± 64.0	0.13	(-0.04;0.29)	0.137	0.472	243.2 ± 53.3	ref.	231.1 ± 68.5	-0.09	(-0.43;0.26)	0.626	0.907	251.9 ± 68.4	0.36	(0.00;0.71)	0.051	0.273	0.060	0.329
BIOGENIC AMINES																			
Creatinine	74.5 ± 17.4	0.03	(-0.10;0.17)	0.630	0.891	76.5 ± 14.8	ref.	77.6 ± 21.2	0.28	(-0.01;0.57)	0.056	0.685	69.3 ± 14.3	0.05	(-0.25;0.35)	0.723	0.866	0.715	0.868
Kynurenine	2.7 ± 0.7	-0.13	(-0.29;0.03)	0.125	0.471	2.7 ± 0.9	ref.	2.7 ± 0.7	-0.05	(-0.39;0.30)	0.792	0.907	2.5 ± 0.5	-0.14	(-0.50;0.21)	0.435	0.715	0.604	0.839
Sarcosine	4.4 ± 1.1	-0.18	(-0.35;-0.01)	0.038	0.252	4.8 ± 1.2	ref.	4.2 ± 0.9	-0.34	(-0.70;0.01)	0.061	0.685	4.2 ± 1.1	-0.41	(-0.78;-0.04)	0.033	0.259	0.020	0.199
Serotonin	0.8 ± 0.6	0.01	(-0.16;0.18)	0.942	0.970	0.8 ± 0.6	ref.	0.8 ± 0.6	-0.28	(-0.64;0.08)	0.125	0.728	0.7 ± 0.5	-0.29	(-0.66;0.08)	0.127	0.371	0.163	0.445
Taurine	120.9 ± 49.4	0.04	(-0.13;0.21)	0.638	0.891	120.6 ± 48.2	ref.	118.8 ± 52.1	-0.23	(-0.59;0.14)	0.223	0.907	123.2 ± 48.4	-0.19	(-0.57;0.19)	0.330	0.604	0.321	0.604

Trans 4- Hydroxyproline Asymmetric dimethylarginine	14-OH- Pro	10.2 ± 5.0	-0.12	(-0.29;0.05)	0.164	0.517	10.6 ± 4.1	ref.	10.1 ± 4.4	0.05	(-0.31;0.40)	0.789	0.907	9.8 ± 6.3	-0.09	(-0.45;0.28)	0.642	0.835	0.602	0.839
	ADMA	0.5 ± 0.1	-0.12	(-0.27;0.04)	0.136	0.472	0.5 ± 0.1	ref.	0.5 ± 0.1	-0.02	(-0.34;0.30)	0.908	0.944	0.4 ± 0.1	-0.28	(-0.61;0.06)	0.106	0.339	0.106	0.369
	SDMA	0.5 ± 0.1	-0.05	(-0.21;0.11)	0.554	0.843	0.5 ± 0.1	ref.	0.5 ± 0.1	0.17	(-0.16;0.50)	0.311	0.907	0.4 ± 0.1	-0.28	(-0.62;0.06)	0.112	0.340	0.116	0.369
GLYCEROPHOSPHOLIPIDS	LysoPC (acyl) C16:0	90.4 ± 20.1	-0.12	(-0.29;0.06)	0.196	0.528	93.2 ± 22.9	ref.	87.3 ± 18.3	-0.20	(-0.57;0.17)	0.297	0.907	90.8 ± 18.7	-0.12	(-0.50;0.26)	0.540	0.786	0.444	0.725
	LysoPC (acyl) C16:1	2.6 ± 0.9	-0.28	(-0.45;-0.11)	0.001	0.056	2.8 ± 1.2	ref.	2.5 ± 0.8	-0.35	(-0.71;0.01)	0.061	0.685	2.4 ± 0.7	-0.44	(-0.82;-0.06)	0.024	0.213	0.018	0.199
	LysoPC (acyl) C17:0	1.6 ± 0.5	0.11	(-0.06;0.28)	0.198	0.528	1.5 ± 0.6	ref.	1.5 ± 0.5	0.16	(-0.19;0.51)	0.380	0.907	1.7 ± 0.4	0.24	(-0.12;0.61)	0.197	0.480	0.247	0.542
	LysoPC (acyl) C18:0	26.4 ± 6.8	-0.04	(-0.21;0.13)	0.643	0.891	26.3 ± 7.9	ref.	25.8 ± 6.4	-0.04	(-0.40;0.32)	0.846	0.919	27 ± 6.1	-0.04	(-0.41;0.34)	0.848	0.936	0.730	0.868
	LysoPC (acyl) C18:1	17.9 ± 5.4	-0.19	(-0.36;-0.02)	0.026	0.208	18.9 ± 6.6	ref.	16.9 ± 4.3	-0.30	(-0.65;0.06)	0.108	0.728	18.0 ± 4.8	-0.19	(-0.57;0.18)	0.312	0.596	0.233	0.542
	LysoPC (acyl) C18:2	29.2 ± 10.4	-0.05	(-0.22;0.12)	0.551	0.843	30.1 ± 11.8	ref.	28.1 ± 10.2	-0.06	(-0.41;0.29)	0.754	0.907	29.6 ± 9.0	0.03	(-0.33;0.39)	0.868	0.936	0.939	0.968
	LysoPC (acyl) C20:3	2.0 ± 0.7	-0.16	(-0.33;0.01)	0.059	0.361	2.2 ± 0.9	ref.	1.9 ± 0.5	-0.28	(-0.64;0.07)	0.120	0.728	2.0 ± 0.6	-0.25	(-0.62;0.12)	0.194	0.480	0.148	0.430
	LysoPC (acyl) C20:4	5.9 ± 1.7	-0.12	(-0.29;0.05)	0.158	0.517	6.2 ± 2.1	ref.	5.7 ± 1.6	-0.11	(-0.48;0.25)	0.536	0.907	5.8 ± 1.4	-0.16	(-0.54;0.22)	0.407	0.686	0.317	0.604
	LysoPC (acyl) C24:0	0.14 ± 0.03	-0.01	(-0.17;0.14)	0.867	0.947	0.13 ± 0.03	ref.	0.13 ± 0.03	-0.08	(-0.41;0.24)	0.618	0.907	0.14 ± 0.03	0.13	(-0.21;0.47)	0.461	0.718	0.539	0.776
	LysoPC (acyl) C28:1	0.3 ± 0.1	0.07	(-0.07;0.21)	0.359	0.707	0.3 ± 0.1	ref.	0.3 ± 0.1	0.12	(-0.17;0.42)	0.419	0.907	0.3 ± 0.1	0.18	(-0.13;0.49)	0.248	0.522	0.254	0.542
	PC (diacyl) C28:1	3.0 ± 0.8	0.10	(-0.06;0.26)	0.212	0.531	2.8 ± 0.7	ref.	3 ± 0.8	0.17	(-0.17;0.50)	0.327	0.907	3.2 ± 0.7	0.31	(-0.04;0.65)	0.085	0.327	0.098	0.369
	PC (diacyl) C30:0	4.0 ± 1.3	-0.04	(-0.21;0.13)	0.634	0.891	4.0 ± 1.5	ref.	3.9 ± 1.2	-0.09	(-0.46;0.27)	0.617	0.907	4.2 ± 1.3	-0.02	(-0.39;0.36)	0.932	0.968	0.961	0.976
	PC (diacyl) C32:0	26.6 ± 5.5	-0.08	(-0.24;0.09)	0.364	0.707	26.5 ± 6.3	ref.	26 ± 4.9	-0.17	(-0.52;0.18)	0.351	0.907	27.4 ± 5.1	-0.02	(-0.39;0.34)	0.898	0.947	0.898	0.960
	PC (diacyl) C32:1	32.0 ± 16.2	-0.21	(-0.39;-0.04)	0.015	0.153	35.2 ± 20.9	ref.	30.5 ± 13.7	-0.29	(-0.65;0.08)	0.123	0.728	30.3 ± 12.4	-0.33	(-0.71;0.04)	0.083	0.327	0.084	0.369
	PC (diacyl) C32:2	8.3 ± 3.1	0.02	(-0.15;0.19)	0.785	0.947	7.9 ± 3.2	ref.	8.6 ± 3.1	0.22	(-0.13;0.58)	0.223	0.907	8.6 ± 3.0	0.07	(-0.30;0.45)	0.694	0.853	0.732	0.868
	PC (diacyl) C32:3	0.9 ± 0.2	0.04	(-0.12;0.19)	0.646	0.891	0.8 ± 0.3	ref.	0.9 ± 0.2	0.17	(-0.16;0.50)	0.308	0.907	1.0 ± 0.2	0.17	(-0.17;0.51)	0.330	0.604	0.403	0.683
	PC (diacyl) C34:1	36.5 ± 82.8	-0.21	(-0.38;-0.04)	0.017	0.164	37.4 ± 96	ref.	350.7 ± 76.4	-0.36	(-0.72;0.00)	0.051	0.685	368.1 ± 73.2	-0.27	(-0.64;0.10)	0.155	0.424	0.143	0.425

PC aa C34:2	739.8 ± 128.1	0.00	(-0.18;0.17)	0.980	0.983	723.8 ± 137	ref.	739.4 ± 127.2	0.07	(-0.29;0.44)	0.702	0.907	756.1 ± 119.3	0.10	(-0.28;0.48)	0.616	0.818	0.668	0.845
PC aa C34:3	30.1 ± 10.2	-0.11	(-0.28;0.06)	0.202	0.528	29.6 ± 10.2	ref.	29.7 ± 9.4	-0.05	(-0.41;0.30)	0.778	0.907	31.1 ± 10.9	-0.11	(-0.48;0.26)	0.569	0.804	0.493	0.754
PC aa C34:4	3.1 ± 1.2	0.01	(-0.16;0.18)	0.904	0.947	3.0 ± 1.3	ref.	3.1 ± 1.1	0.18	(-0.17;0.54)	0.314	0.907	3.2 ± 1.1	0.07	(-0.30;0.44)	0.704	0.858	0.769	0.880
PC aa C36:0	3.2 ± 1.0	0.10	(-0.07;0.26)	0.243	0.593	3.1 ± 1.2	ref.	3.2 ± 0.9	0.18	(-0.17;0.53)	0.312	0.907	3.4 ± 1.0	0.30	(-0.07;0.66)	0.110	0.340	0.135	0.411
PC aa C36:1	62.9 ± 17.8	-0.13	(-0.29;0.04)	0.130	0.471	63.3 ± 19.9	ref.	60.20 ± 15.3	-0.20	(-0.54;0.14)	0.252	0.907	65.3 ± 17.7	-0.14	(-0.49;0.22)	0.454	0.716	0.431	0.713
PC aa C36:2	384.7 ± 79.7	0.02	(-0.15;0.18)	0.836	0.947	369.3 ± 79.1	ref.	386.6 ± 82.6	0.13	(-0.21;0.48)	0.444	0.907	398.3 ± 75.7	0.08	(-0.28;0.44)	0.665	0.849	0.719	0.868
PC aa C36:3	192.6 ± 43.8	-0.08	(-0.25;0.08)	0.321	0.682	191.9 ± 49.8	ref.	188.7 ± 38.9	-0.08	(-0.43;0.26)	0.636	0.907	197.2 ± 42.2	-0.11	(-0.47;0.25)	0.538	0.786	0.501	0.754
PC aa C36:4	291.5 ± 72.0	-0.03	(-0.19;0.14)	0.742	0.947	289.8 ± 76.5	ref.	290.20 ± 71.2	0.04	(-0.31;0.39)	0.810	0.919	294.4 ± 69.2	-0.03	(-0.39;0.33)	0.871	0.936	0.804	0.912
PC aa C36:5	45.8 ± 27.8	0.03	(-0.14;0.20)	0.744	0.947	46.8 ± 36.4	ref.	42.2 ± 20.4	-0.02	(-0.38;0.34)	0.930	0.944	48.2 ± 24	0.22	(-0.15;0.59)	0.249	0.522	0.275	0.562
PC aa C36:6	1.5 ± 0.6	0.13	(-0.04;0.30)	0.129	0.471	1.4 ± 0.7	ref.	1.5 ± 0.6	0.21	(-0.14;0.56)	0.243	0.907	1.7 ± 0.7	0.37	(0.01;-0.74)	0.046	0.263	0.064	0.329
PC aa C38:0	2.9 ± 0.9	0.08	(-0.08;0.25)	0.312	0.675	2.7 ± 1.0	ref.	2.8 ± 0.7	0.15	(-0.19;0.49)	0.389	0.907	3.1 ± 0.8	0.32	(-0.04;0.67)	0.079	0.327	0.089	0.369
PC aa C38:3	53.2 ± 13.6	-0.11	(-0.27;0.04)	0.158	0.517	52.9 ± 14.1	ref.	52.1 ± 12.5	-0.16	(-0.49;0.16)	0.330	0.907	54.6 ± 14.3	-0.22	(-0.56;0.12)	0.198	0.480	0.194	0.490
PC aa C38:4	131.5 ± 35.0	-0.05	(-0.20;0.11)	0.562	0.845	130 ± 39.0	ref.	132.1 ± 33	0.06	(-0.27;0.38)	0.738	0.907	132.5 ± 33.1	-0.12	(-0.46;0.22)	0.479	0.737	0.456	0.737
PC aa C38:5	66.2 ± 19.0	-0.07	(-0.23;0.09)	0.401	0.729	66.4 ± 21.8	ref.	63.9 ± 16.5	-0.12	(-0.46;0.22)	0.500	0.907	68.4 ± 18.5	-0.05	(-0.40;0.31)	0.798	0.930	0.738	0.868
PC aa C38:6	101.1 ± 33.6	0.13	(-0.03;0.30)	0.124	0.471	95.7 ± 36.4	ref.	97.4 ± 27.3	0.12	(-0.23;0.47)	0.508	0.907	110.20 ± 35	0.38	(0.02;-0.74)	0.039	0.263	0.053	0.329
PC aa C40:2	0.3 ± 0.1	-0.08	(-0.24;0.08)	0.346	0.707	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.15	(-0.50;0.19)	0.381	0.907	0.3 ± 0.1	0.03	(-0.33;0.39)	0.875	0.936	0.939	0.968
PC aa C40:3	0.4 ± 0.1	-0.13	(-0.29;0.03)	0.110	0.471	0.4 ± 0.2	ref.	0.3 ± 0.1	-0.36	(-0.70;-0.03)	0.034	0.685	0.4 ± 0.1	-0.16	(-0.50;0.19)	0.378	0.649	0.329	0.604
PC aa C40:4	3.0 ± 0.8	-0.22	(-0.39;-0.06)	0.010	0.113	3.2 ± 1.1	ref.	2.9 ± 0.6	-0.39	(-0.74;-0.04)	0.031	0.685	2.9 ± 0.7	-0.54	(-0.90;-0.18)	0.004	0.091	0.004	0.098
PC aa C40:5	8.3 ± 2.4	-0.19	(-0.35;-0.02)	0.025	0.208	8.6 ± 2.9	ref.	8.1 ± 2.1	-0.27	(-0.62;0.08)	0.130	0.728	8.1 ± 2.3	-0.42	(-0.78;-0.06)	0.023	0.213	0.024	0.203
PC aa C40:6	28.3 ± 10.2	0.07	(-0.09;0.23)	0.403	0.729	27.1 ± 11.6	ref.	27.5 ± 7.8	0.07	(-0.28;0.41)	0.694	0.907	30.3 ± 10.7	0.22	(-0.14;0.58)	0.233	0.512	0.246	0.542
PC aa C42:0	0.3 ± 0.1	0.06	(-0.10;0.22)	0.463	0.774	0.3 ± 0.1	ref.	0.3 ± 0.1	0.13	(-0.21;0.46)	0.450	0.907	0.4 ± 0.1	0.29	(-0.06;0.64)	0.102	0.338	0.116	0.369
PC aa C42:1	0.18 ± 0.05	0.09	(-0.07;0.24)	0.291	0.651	0.17 ± 0.05	ref.	0.17 ± 0.04	0.11	(-0.22;0.45)	0.509	0.907	0.2 ± 0.1	0.31	(-0.03;0.66)	0.077	0.327	0.089	0.369

PC (diacyl) C42:2	PC aa C42:2	0.16 ± 0.04	0.02	(-0.14;0.19)	0.790	0.947	0.15 ± 0.05	ref.	0.15 ± 0.04	0.12	(-0.22;0.47)	0.482	0.907	0.16 ± 0.03	0.25	(-0.11;-0.61)	0.169	0.443	0.189	0.487
PC (diacyl) C42:4	PC aa C42:4	0.10 ± 0.02	-0.09	(-0.26;0.08)	0.291	0.651	0.10 ± 0.03	ref.	0.11 ± 0.03	-0.04	(-0.40;0.32)	0.826	0.919	0.10 ± 0.02	-0.26	(-0.64;-0.11)	0.175	0.450	0.182	0.479
PC (diacyl) C42:5	PC aa C42:5	0.3 ± 0.1	-0.10	(-0.27;0.07)	0.266	0.615	0.3 ± 0.2	ref.	0.2 ± 0.1	-0.26	(-0.62;0.10)	0.159	0.812	0.3 ± 0.1	-0.14	(-0.52;-0.23)	0.448	0.715	0.464	0.740
PC (diacyl) C42:6	PC aa C42:6	0.4 ± 0.1	-0.15	(-0.32;0.01)	0.071	0.388	0.4 ± 0.2	ref.	0.3 ± 0.1	-0.40	(-0.76;-0.05)	0.025	0.685	0.4 ± 0.1	-0.30	(-0.67;-0.06)	0.103	0.338	0.096	0.369
PC (acyl-alkyl) C30:0	PC ae C30:0	0.3 ± 0.1	0.02	(-0.15;0.18)	0.825	0.947	0.3 ± 0.1	ref.	0.3 ± 0.1	0.02	(-0.33;0.37)	0.911	0.944	0.4 ± 0.1	0.09	(-0.27;-0.45)	0.640	0.835	0.652	0.845
PC (acyl-alkyl) C30:2	PC ae C30:2	0.08 ± 0.02	0.20	(0.06;0.34)	0.006	0.098	0.07 ± 0.02	ref.	0.09 ± 0.02	0.42	(0.13;0.72)	0.006	0.685	0.09 ± 0.02	0.50	(0.2;-0.81)	0.002	0.091	0.002	0.098
PC (acyl-alkyl) C32:1	PC ae C32:1	5.3 ± 1.2	-0.02	(-0.18;0.15)	0.841	0.947	5.0 ± 1.2	ref.	5.3 ± 1.3	0.09	(-0.26;0.44)	0.613	0.907	5.4 ± 1.1	0.10	(-0.26;0.46)	0.586	0.804	0.613	0.839
PC (acyl-alkyl) C32:2	PC ae C32:2	1.4 ± 0.4	0.01	(-0.14;0.17)	0.873	0.947	1.3 ± 0.4	ref.	1.4 ± 0.4	0.17	(-0.16;0.50)	0.321	0.907	1.4 ± 0.3	0.19	(-0.16;0.53)	0.294	0.571	0.321	0.604
PC (acyl-alkyl) C34:0	PC ae C34:0	2.5 ± 0.6	0.06	(-0.11;0.22)	0.484	0.782	2.3 ± 0.7	ref.	2.4 ± 0.6	0.08	(-0.26;0.43)	0.641	0.907	2.6 ± 0.6	0.20	(-0.16;0.56)	0.272	0.537	0.247	0.542
PC (acyl-alkyl) C34:1	PC ae C34:1	18.0 ± 3.9	-0.06	(-0.22;0.10)	0.471	0.774	17.4 ± 4.1	ref.	18.0 ± 4.0	-0.05	(-0.38;0.29)	0.788	0.907	18.8 ± 3.4	0.01	(-0.34;0.35)	0.968	0.968	0.955	0.976
PC (acyl-alkyl) C34:2	PC ae C34:2	20.2 ± 5.3	0.01	(-0.15;0.17)	0.926	0.962	19.3 ± 5.7	ref.	20.5 ± 5.4	0.18	(-0.16;0.52)	0.307	0.907	20.9 ± 4.8	0.08	(-0.27;-0.44)	0.650	0.838	0.713	0.868
PC (acyl-alkyl) C34:3	PC ae C34:3	13.1 ± 4.0	0.05	(-0.12;0.21)	0.570	0.849	12.4 ± 3.7	ref.	13.1 ± 4.2	0.13	(-0.21;0.47)	0.461	0.907	13.9 ± 3.8	0.23	(-0.12;-0.59)	0.204	0.480	0.282	0.562
PC (acyl-alkyl) C36:0	PC ae C36:0	1.2 ± 0.3	-0.15	(-0.32;0.02)	0.082	0.392	1.2 ± 0.4	ref.	1.1 ± 0.2	-0.31	(-0.67;0.04)	0.088	0.728	1.2 ± 0.2	-0.19	(-0.56;-0.18)	0.322	0.604	0.339	0.604
PC (acyl-alkyl) C36:1	PC ae C36:1	11.5 ± 2.7	0.07	(-0.08;0.23)	0.356	0.707	10.8 ± 2.7	ref.	11.4 ± 2.7	0.13	(-0.21;0.46)	0.452	0.907	12.2 ± 2.5	0.21	(-0.13;-0.56)	0.226	0.511	0.230	0.542
PC (acyl-alkyl) C36:2	PC ae C36:2	21.7 ± 5.4	0.16	(0.01;0.32)	0.036	0.252	19.9 ± 5.5	ref.	22.0 ± 5.4	0.31	(-0.01;0.64)	0.057	0.685	23.3 ± 4.8	0.35	(0.01;0.68)	0.043	0.263	0.052	0.329
PC (acyl-alkyl) C36:3	PC ae C36:3	11.4 ± 2.8	-0.06	(-0.22;0.10)	0.463	0.774	11.1 ± 3	ref.	11.4 ± 2.9	0.05	(-0.29;0.39)	0.782	0.907	11.7 ± 2.6	-0.04	(-0.39;0.32)	0.841	0.936	0.759	0.880
PC (acyl-alkyl) C36:4	PC ae C36:4	26.3 ± 6.4	-0.07	(-0.24;0.10)	0.412	0.736	26.2 ± 6.9	ref.	26.1 ± 5.4	0.02	(-0.34;0.38)	0.918	0.944	26.4 ± 6.9	-0.10	(-0.47;-0.27)	0.597	0.804	0.532	0.775
PC (acyl-alkyl) C36:5	PC ae C36:5	18.3 ± 5.0	0.01	(-0.15;0.17)	0.899	0.947	17.8 ± 5.4	ref.	18.4 ± 4.4	0.20	(-0.14;0.55)	0.246	0.907	18.8 ± 5.1	0.14	(-0.22;0.50)	0.447	0.715	0.524	0.775
PC (acyl-alkyl) C38:0	PC ae C38:0	2.5 ± 0.8	0.11	(-0.06;0.27)	0.205	0.528	2.4 ± 0.9	ref.	2.4 ± 0.7	0.12	(-0.22;0.47)	0.483	0.907	2.7 ± 0.8	0.36	(0.01;0.72)	0.047	0.263	0.074	0.354
PC (acyl-alkyl) C38:2	PC ae C38:2	2.2 ± 0.6	0.01	(-0.15;0.17)	0.890	0.947	2.1 ± 0.6	ref.	2.2 ± 0.6	0.14	(-0.20;0.48)	0.435	0.907	2.3 ± 0.5	0.07	(-0.28;0.43)	0.686	0.851	0.716	0.868
PC (acyl-alkyl) C38:3	PC ae C38:3	4.8 ± 1.1	0.06	(-0.09;0.22)	0.423	0.746	4.5 ± 1.1	ref.	4.8 ± 1.1	0.13	(-0.20;0.45)	0.442	0.907	5.0 ± 1.1	0.16	(-0.18;0.50)	0.359	0.625	0.361	0.629
PC (acyl-alkyl) C38:4	PC ae C38:4	17.2 ± 3.6	0.00	(-0.16;0.16)	0.983	0.983	16.5 ± 3.5	ref.	17.4 ± 3.5	0.14	(-0.20;0.49)	0.410	0.907	17.7 ± 3.9	0.01	(-0.34;-0.37)	0.943	0.968	0.997	0.997

PC (acyl-alkyl) C38:5 C38:5	PC ae C38:5	21.9 ± 4.7	-0.09	(-0.25;0.07)	0.263	0.615	21.7 ± 5.0	ref.	21.8 ± 4.1	0.03	(-0.31;0.38)	0.850	0.919	22.3 ± 4.9	-0.08	(-0.43;0.28)	0.678	0.849	0.611	0.839
PC (acyl-alkyl) C38:6 C38:6	PC ae C38:6	9.2 ± 2.6	0.08	(-0.08;0.24)	0.352	0.707	8.7 ± 3.0	ref.	9.2 ± 2.2	0.24	(-0.10;0.58)	0.164	0.812	9.8 ± 2.5	0.30	(-0.05;0.65)	0.092	0.338	0.110	0.369
PC (acyl-alkyl) C40:1 C40:1	PC ae C40:1	1.1 ± 0.3	0.03	(-0.13;0.20)	0.700	0.919	1.1 ± 0.3	ref.	1.1 ± 0.3	0.10	(-0.26;0.45)	0.592	0.907	1.2 ± 0.3	0.23	(-0.14;0.59)	0.224	0.511	0.285	0.562
PC (acyl-alkyl) C40:2 C40:2	PC ae C40:2	1.6 ± 0.4	0.14	(-0.02;0.30)	0.078	0.388	1.5 ± 0.4	ref.	1.5 ± 0.4	0.09	(-0.25;0.42)	0.610	0.907	1.7 ± 0.4	0.39	(0.04;0.74)	0.029	0.240	0.031	0.243
PC (acyl-alkyl) C40:3 C40:3	PC ae C40:3	0.8 ± 0.2	-0.01	(-0.17;0.14)	0.860	0.947	0.8 ± 0.2	ref.	0.8 ± 0.2	-0.04	(-0.36;0.28)	0.791	0.907	0.9 ± 0.2	0.09	(-0.24;0.42)	0.595	0.804	0.668	0.845
PC (acyl-alkyl) C40:4 C40:4	PC ae C40:4	2.2 ± 0.4	-0.02	(-0.18;0.14)	0.767	0.947	2.1 ± 0.4	ref.	2.2 ± 0.4	-0.05	(-0.39;0.29)	0.762	0.907	2.3 ± 0.5	-0.01	(-0.36;0.34)	0.953	0.968	0.927	0.968
PC (acyl-alkyl) C40:5 C40:5	PC ae C40:5	3.4 ± 0.7	-0.01	(-0.17;0.15)	0.900	0.947	3.2 ± 0.6	ref.	3.4 ± 0.7	0.05	(-0.29;0.38)	0.789	0.907	3.5 ± 0.7	0.10	(-0.25;0.44)	0.591	0.804	0.610	0.839
PC (acyl-alkyl) C40:6 C40:6	PC ae C40:6	4.8 ± 1.2	0.13	(-0.03;0.29)	0.102	0.471	4.4 ± 1.3	ref.	4.7 ± 1.1	0.26	(-0.07;0.59)	0.129	0.728	5.2 ± 1.3	0.42	(0.07;0.76)	0.019	0.195	0.022	0.199
PC (acyl-alkyl) C42:1 C42:1	PC ae C42:1	0.3 ± 0.1	-0.15	(-0.30;0.01)	0.075	0.388	0.3 ± 0.1	ref.	0.3 ± 0.1	-0.39	(-0.72;-0.05)	0.024	0.685	0.3 ± 0.1	-0.26	(-0.60;0.09)	0.149	0.417	0.100	0.369
PC (acyl-alkyl) C42:2 C42:2	PC ae C42:2	0.4 ± 0.1	0.02	(-0.13;0.18)	0.759	0.947	0.4 ± 0.1	ref.	0.4 ± 0.1	-0.14	(-0.46;0.19)	0.408	0.907	0.5 ± 0.1	0.19	(-0.15;0.53)	0.271	0.537	0.327	0.604
PC (acyl-alkyl) C42:3 C42:3	PC ae C42:3	0.6 ± 0.1	0.04	(-0.12;0.21)	$\frac{0.584}{7}$	0.861	0.5 ± 0.1	ref.	0.5 ± 0.1	-0.07	(-0.40;0.27)	$\frac{0.697}{5}$	0.907	0.6 ± 0.2	0.27	(-0.07;0.62)	0.1238	0.369	0.182	0.479
PC (acyl-alkyl) C42:4 C42:4	PC ae C42:4	0.7 ± 0.2	-0.04	(-0.20;0.12)	0.652	0.891	0.7 ± 0.1	ref.	0.7 ± 0.2	-0.10	(-0.44;0.25)	0.582	0.907	0.7 ± 0.2	0.01	(-0.35;0.36)	0.964	0.968	0.994	0.997
PC (acyl-alkyl) C42:5 C42:5	PC ae C42:5	1.5 ± 0.3	-0.02	(-0.19;0.14)	0.797	0.947	1.5 ± 0.3	ref.	1.5 ± 0.3	0.04	(-0.31;0.38)	0.841	0.919	1.6 ± 0.4	0.10	(-0.26;0.45)	0.600	0.804	0.660	0.845
PC (acyl-alkyl) C44:3 C44:3	PC ae C44:3	0.07 ± 0.02	-0.11	(-0.27;0.05)	0.170	0.517	0.07 ± 0.02	ref.	0.07 ± 0.02	-0.21	(-0.54;0.12)	0.217	0.907	0.07 ± 0.02	-0.17	(-0.52;0.18)	0.334	0.604	0.343	0.604
PC (acyl-alkyl) C44:4 C44:4	PC ae C44:4	0.2 ± 0.1	0.01	(-0.15;0.17)	0.899	0.947	0.2 ± 0.1	ref.	0.2 ± 0.1	-0.06	(-0.40;0.29)	0.743	0.907	0.2 ± 0.1	0.17	(-0.19;0.52)	0.359	0.625	0.416	0.697
PC (acyl-alkyl) C44:5 C44:5	PC ae C44:5	1.0 ± 0.3	-0.06	(-0.23;0.10)	0.456	0.774	1.0 ± 0.3	ref.	1.0 ± 0.3	-0.10	(-0.45;0.26)	0.597	0.907	1.0 ± 0.3	-0.05	(-0.41;0.32)	0.810	0.936	0.766	0.880
PC (acyl-alkyl) C44:6 C44:6	PC ae C44:6	0.8 ± 0.2	-0.02	(-0.18;0.14)	0.811	0.947	0.8 ± 0.2	ref.	0.8 ± 0.2	0.03	(-0.31;0.37)	0.848	0.919	0.8 ± 0.2	0.10	(-0.25;0.45)	0.589	0.804	0.665	0.845
SPHINGOLIPIDS																				
Hydroxysphingomyelin C14:1	SM OH C14:1	4.0 ± 1.0	0.20	(0.06;0.35)	0.007	0.098	3.5 ± 0.9	ref.	4.0 ± 1.1	0.27	(-0.03;0.58)	0.076	0.728	4.4 ± 1.0	0.50	(0.18;0.81)	0.002	0.091	0.002	0.098
Hydroxysphingomyelin C16:1	SM OH C16:1	1.9 ± 0.5	0.22	(0.08;0.37)	0.004	0.095	1.6 ± 0.4	ref.	1.8 ± 0.5	0.26	(-0.06;0.57)	0.110	0.728	2.1 ± 0.5	0.51	(0.18;0.83)	0.003	0.091	0.002	0.098
Hydroxysphingomyelin C22:1	SM OH C22:1	4.4 ± 1.1	0.20	(0.05;0.35)	0.010	0.113	4.0 ± 1.1	ref.	4.4 ± 1.0	0.29	(-0.03;0.61)	0.081	0.728	4.8 ± 1.0	0.43	(0.1;0.76)	0.013	0.168	0.014	0.188

Hydroxysphingomyelin C22:2	SM OH	3.3 ± 0.9	0.20	(0.06;0.34)	0.007	0.098	2.9 ± 0.8	ref.	3.3 ± 0.9	0.30	(0.00;0.60)	0.053	0.685	3.7 ± 0.8	0.46	(0.15;0.77)	0.004	0.091	0.006	0.118
Hydroxysphingomyelin C24:1	SM OH	0.3 ± 0.1	0.19	(0.03;0.35)	0.025	0.208	0.3 ± 0.1	ref.	0.3 ± 0.1	0.14	(-0.21;0.48)	0.439	0.907	0.3 ± 0.1	0.36	(0.01;0.72)	0.047	0.263	0.061	0.329
Sphingomyelin C16:0	SM	67.2 ± 13.6	-0.02	(-0.17;0.13)	0.790	0.947	65.5 ± 14.8	ref.	65.3 ± 13.8	-0.16	(-0.48;0.16)	0.324	0.907	70.6 ± 11.6	0.06	(-0.27;0.39)	0.715	0.863	0.846	0.922
Sphingomyelin C16:1	SM	9.5 ± 2.2	0.06	(-0.08;0.21)	0.386	0.720	8.8 ± 2.0	ref.	9.4 ± 2.5	0.10	(-0.21;0.40)	0.536	0.907	10.1 ± 1.9	0.23	(-0.08;0.55)	0.144	0.411	0.226	0.542
Sphingomyelin C18:0	SM	12.0 ± 3.3	0.06	(-0.10;0.22)	0.474	0.774	11.6 ± 3.8	ref.	11.6 ± 3.1	-0.08	(-0.41;0.25)	0.640	0.907	12.9 ± 2.7	0.20	(-0.14;0.55)	0.253	0.522	0.281	0.562
Sphingomyelin C18:1	SM	5.7 ± 1.6	0.10	(-0.05;0.25)	0.189	0.528	5.2 ± 1.6	ref.	5.6 ± 1.8	0.07	(-0.24;0.38)	0.645	0.907	6.2 ± 1.4	0.30	(-0.03;0.62)	0.075	0.327	0.100	0.369
Sphingomyelin C20:2	SM	0.2 ± 0.1	0.08	(-0.07;0.23)	0.298	0.654	0.14 ± 0.05	ref.	0.15 ± 0.05	0.17	(-0.15;0.49)	0.304	0.907	0.2 ± 0.1	0.13	(-0.20;0.47)	0.445	0.715	0.477	0.751
Sphingomyelin C24:0	SM	6.1 ± 1.4	0.02	(-0.14;0.19)	0.771	0.947	6.0 ± 1.5	ref.	6.0 ± 1.4	-0.04	(-0.38;0.30)	0.826	0.919	6.4 ± 1.3	0.11	(-0.25;0.46)	0.558	0.804	0.662	0.845
Sphingomyelin C24:1	SM	13.9 ± 3.4	-0.05	(-0.21;0.11)	0.554	0.843	14 ± 4.4	ref.	13.3 ± 3.1	-0.19	(-0.53;0.15)	0.273	0.907	14.4 ± 2.5	0.01	(-0.34;0.36)	0.955	0.968	0.899	0.960
Sphingomyelin C26:0	SM	0.04 ± 0.01	0.15	(-0.02;0.32)	0.076	0.388	0.03 ± 0.01	ref.	0.04 ± 0.01	0.00	(-0.35;0.35)	0.996	0.998	0.04 ± 0.01	0.31	(-0.06;0.67)	0.101	0.338	0.155	0.433
Sphingomyelin C26:1	SM	0.10 ± 0.04	0.08	(-0.09;0.25)	0.369	0.707	0.1 ± 0.1	ref.	0.10 ± 0.03	0.15	(-0.21;0.50)	0.420	0.907	0.11 ± 0.03	0.33	(-0.04;0.70)	0.083	0.327	0.102	0.369
HEXOSES																				
Hexoses	H1	5574.0 ± 1481.3	-0.01	(-0.18;0.16)	0.897	0.947	5575.9 ± 1303.6	ref.	5451.5 ± 1480.2	-0.11	(-0.47;0.25)	0.543	0.907	5694.4 ± 1652.4	0.04	(-0.34;0.41)	0.845	0.936	0.841	0.922

Bold metabolites and corresponding p-values are significantly associated with colorectal cancer stage after FDR correction; ¹Tested using multiple linear regression models analyzing associations of dietary exposures (continuous and in tertiles) as independent variable and log transformed Z-standardized metabolite concentrations as dependent variable. The continuous analysis is presented per one-point, ten-points, SD increase for the WCRF dietary score, DHD15-index, and dietary patterns, respectively. Tertile cut-off scores were 1.75 and 2.25 for the WCRF dietary score and 68.3 and 78.9 for the DHD15-index. Tertile cut-off scores were -0.3 and 0.5, -0.1 and 0.4, and -0.3 and 0.5 for the Western, Carnivore, and Prudent pattern, respectively. Regression models were adjusted for sex, age, analytical batch, body mass index (continuous), smoking status, a nd stage; ¹untransformed and unadjusted metabolite concentrations in µmol/l; ²Confidence Interval; ³p-value corrected for False Discovery Rate.

Supplementary Table S3 Baseline characteristics by diet quality index and dietary pattern, comparing the lowest (T1) with the highest tertile (T3)

	<i>WCRF dietary score</i>		<i>DHD15-index</i>		<i>Western pattern</i>		<i>Carnivore pattern</i>		<i>Prudent pattern</i>	
	<i>T1</i>	<i>T3</i>	<i>T1</i>	<i>T3</i>	<i>T1</i>	<i>T3</i>	<i>T1</i>	<i>T3</i>	<i>T1</i>	<i>T3</i>
Number of participants	77	62	65	65	65	65	65	65	65	65
Male sex, n (%)	51 (66)	32 (52)	47 (72)	36 (55)	35 (54)	43 (66)	31 (48)	45 (69)	52 (80)	26 (40)
Age	65.5 ± 8.3	66.7 ± 10.1	64.0 ± 9.5	67.4 ± 9.5	69.5 ± 9.2	62.1 ± 8.4	65.4 ± 10.2	67.2 ± 8.2	65.1 ± 8.0	66.2 ± 9.0
Body mass index (kg/m ²)	25.0 ± 5.6	26.0 ± 5.2	26.4 ± 3.8	25.0 ± 5.7	26.2 ± 3.6	25.4 ± 6.0	23.7 ± 6.2	27.0 ± 4.0	26.6 ± 4.0	24.5 ± 5.7
Underweight, <18.5, n (%)	2 (3)	1 (2)	0 (0)	2 (3)	0 (0)	2 (3)	3 (5)	0 (0)	0 (0)	2 (3)
Normal weight, 18.5-24.9, n (%)	36 (47)	26 (42)	27 (42)	27 (42)	28 (43)	26 (40)	34 (52)	26 (40)	26 (40)	31 (48)
Overweight, 25-29.9, n (%)	27 (35)	24 (39)	27 (42)	29 (45)	26 (40)	27 (42)	23 (35)	26 (40)	25 (39)	26 (40)
Obese, ≥30, n (%)	12 (16)	11 (18)	11 (17)	7 (11)	11 (17)	10 (15)	5 (8)	13 (20)	14 (22)	6 (9)
Smoking, n (%)										
Current	11 (14)	7 (11)	15 (23)	1 (2)	6 (9)	8 (12)	9 (14)	6 (9)	12 (19)	2 (3)
Former	49 (64)	37 (60)	36 (55)	42 (65)	34 (52)	40 (62)	31 (48)	44 (68)	39 (60)	41 (63)
Never	17 (22)	18 (29)	14 (22)	22 (34)	25 (39)	17 (26)	25 (39)	15 (23)	14 (22)	22 (34)
Stage, n (%)										
I	20 (26)	18 (29)	14 (22)	22 (34)	17 (26)	15 (23)	22 (34)	12 (19)	20 (31)	17 (26)
II	24 (31)	18 (29)	23 (35)	20 (31)	21 (32)	18 (28)	20 (31)	24 (37)	23 (35)	23 (35)
III	29 (38)	24 (39)	25 (39)	20 (31)	25 (39)	29 (45)	21 (32)	27 (42)	19 (29)	24 (37)
IV	4 (5)	2 (3)	3 (5)	3 (5)	2 (3)	3 (5)	2 (3)	2 (3)	3 (5)	1 (2)
Tumor site ¹ , n (%)										
Colon – proximal	23 (30)	15 (24)	17 (26)	14 (22)	21 (32)	14 (22)	21 (32)	22 (34)	21 (32)	16 (25)
Colon – distal	26 (34)	25 (40)	20 (31)	30 (46)	21 (32)	24 (37)	25 (39)	22 (34)	20 (31)	28 (43)
Rectal	28 (26)	22 (36)	28 (43)	21 (32)	23 (35)	27 (42)	19 (29)	21 (32)	24 (37)	21 (32)
Treatment, n (%)										
Surgery	76 (99)	62 (100)	64 (99)	65 (100)	64 (99)	65 (100)	64 (99)	65 (100)	64 (99)	65 (100)
Neo-adjutant treatment	24 (31)	19 (31)	25 (39)	18 (28)	20 (31)	23 (35)	17 (26)	20 (31)	21 (32)	20 (31)
Total energy intake (kcal/d)	1953 ± 573	1777 ± 547	1764 ± 531	1947 ± 530	1593 ± 481	2061 ± 602	1894 ± 570	1749 ± 540	1970 ± 612	1803 ± 528
Total WCRF dietary score ²	1.4 ± 0.3	2.8 ± 0.4	1.7 ± 0.6	2.4 ± 0.6	2.4 ± 0.7	1.7 ± 0.5	2.0 ± 0.7	2.1 ± 0.5	1.8 ± 0.6	2.4 ± 0.7
Total DHD15-index ³	66.0 ± 11.6	80.7 ± 14.8	58.6 ± 6.6	89.2 ± 8.5	74.9 ± 12.8	71.2 ± 14.6	73.6 ± 13.8	73.4 ± 13.9	64.6 ± 11.1	84.0 ± 12.0

Numbers are presented as mean ± SD or median (IQR) unless mentioned otherwise; ¹Proximal consisting of: hepatic flexure, transverse colon, cecum, appendix, ascending colon; Distal consisting of: descending colon, sigmoid colon, splenic flexure; Rectal consisting of: rectosigmoid junction, rectum; ²Adherence to the dietary recommendations of the World Cancer Research Fund (WCRF), ranged 0 to 5 [18]; ³Adherence to the Dutch Healthy Diet guidelines 2015 (DHD15), ranged 0 to 130 [19].

5

Chapter 5

Circulating folate species and colorectal cancer recurrence and survival

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Abstract

Background Folates, including folic acid, may play a dual role in colorectal cancer development. Folate is suggested to be protective in early carcinogenesis but could accelerate growth of premalignant lesions or micrometastases. Whether circulating concentrations of folate and folic acid, measured around time of diagnosis, are associated with recurrence and survival in colorectal cancer patients is largely unknown.

Methods Circulating concentrations of folate, folic acid, and folate catabolites p-aminobenzoylglutamate (pABG) and p-acetamidobenzoylglutamate (apABG) were measured by liquid chromatography–tandem mass spectrometry at diagnosis in 2024 stage I-III colorectal cancer patients from European and U.S. patient cohort studies. Multivariable-adjusted Cox proportional hazard models were used to assess associations between folate, folic acid, and folate catabolites concentrations with recurrence, overall and disease-free survival.

Results No statistically significant associations were observed between folate, pABG, and apABG concentrations and recurrence, overall and disease-free survival, with hazard ratios (HRs) ranging from 0.92 to 1.16. The detection of folic acid in the circulation (yes/no) was not associated with any outcome. However, among patients with detectable folic acid concentrations (n=296), a higher risk of recurrence was observed for each two-fold increase in folic acid (HR (95% confidence interval (95%CI): 1.31 (1.02 to 1.58)). No significant associations were found between folic acid concentrations and overall and disease-free survival.

Conclusions Circulating folate and folate catabolites concentrations at colorectal cancer diagnosis were not associated with recurrence and survival. However, caution is warranted for high blood concentrations of folic acid as they may increase the risk of colorectal cancer recurrence.

Keywords: Colorectal cancer, recurrence, survival, folate, folic acid, folate catabolites, blood concentrations

Introduction

Folates have been hypothesized to play a dual role in relation to colorectal cancer risk [1-3]. Epidemiologic evidence suggests that sufficient folate may protect against colorectal cancer development [1, 4], which is supported by *in vitro* and animal studies [2, 5-7]. However, high circulating concentrations of folate, including folic acid, may facilitate the growth of premalignant lesions or micrometastases once they have been established [2, 8-11].

Circulating concentrations of folates originate from dietary folate naturally occurring in for example green leafy vegetables, legumes or liver products [12], are formed by microbiota present in the gut [13], or originate from the ingestion of the synthetic form of folate, i.e. folic acid, present in dietary supplements and fortified foods [6, 14]. Dietary supplements are regularly consumed by cancer patients [15] and fortification is mandatory in countries such as the U.S. [16]. Folic acid is converted to the active form of folate by the enzyme dihydrofolate reductase (DHFR) [6]. Whenever folic acid cannot be converted to folate by DHFR, the rate-limiting step in folate-mediated one-carbon metabolism (FOCM) [17], due to low DHFR activity or excessive intake of folic acid, unmetabolized folic acid may be detected in the circulation [6].

Some folate is degraded, mainly in the liver, to the catabolite p-aminobenzoylglutamate (pABG) [18], while around 80% is converted to p-acetamidobenzoylglutamate (apABG) in the kidneys [19, 20]. Both catabolites are thought to reflect folate turnover [21, 22] and provide useful insights into the role of folate in health [20]. Folate is crucial in FOCM for nucleotide synthesis as well as DNA repair and, indirectly, for the formation of the methyl donor S-adenosylmethionine involved in DNA methylation [23-25]. Low concentrations of folate may result in aberrant DNA methylation patterns and impaired DNA stability and synthesis, which are defects that may contribute to colorectal carcinogenesis [26, 27].

Although the role of folate in colorectal cancer etiology has been studied extensively, research investigating the association between different folates and colorectal cancer prognosis is scarce. Folic acid not only has different bioavailability but may also possess different biochemical effects compared to natural folates. Folic acid and natural folates differ with respect to affinity for the folate receptors and, therefore, transport and utilization in FOCM [28]. We hypothesize that specifically unmetabolized folic acid may foster growth of remaining cancerous cells after treatment and therefore higher concentrations of folic acid

could lead to more recurrences.

Initial studies among colorectal cancer survivors observed no statistically significant associations between serum folate concentrations and recurrence or survival [29-31]. A study among 78 stage II-IV colorectal cancer patients receiving chemotherapy showed non-significantly fewer colorectal cancer recurrences and deaths with high compared to low serum folate concentrations at diagnosis [29]. Serum folate concentrations at colorectal cancer diagnosis in 93 patients with stage IV disease yielded no association with overall survival [30]. These studies were, however, limited by a modest sample size [29-31] and the relatively small number of events [29, 30]. To the best of our knowledge, no studies have been conducted investigating the association between the different forms of circulating folate and folate catabolites and colorectal cancer recurrence or survival.

Therefore, the aim of the present study was to investigate the association of circulating concentrations of folate, folic acid, and folate catabolites at diagnosis, with recurrence, overall and disease-free survival in 2024 stage I-III colorectal cancer patients originating from six European and U.S. patient cohorts.

Methods

Study population and data collection

A total of 2024 stage I-III colorectal cancer patients from six patient cohorts were included in the current study as part of the international, prospective FOCUS consortium. The FOCUS consortium comprises patients from the COLON study (n=1094, Wageningen University & Research, the Netherlands) (ClinicalTrials.gov Identifier: NCT03191110) [32], the EnCoRe study (n=297, Maastricht University, the Netherlands) [33] (Netherlands Trial Register: 7099), the CORSA study (n=209, Medical University of Vienna, Austria) and three sites of the ColoCare study (n=260, University of Heidelberg and the German Cancer Research Center and National Center for Tumor Diseases, Germany; n=46, Huntsman Cancer Institute, U.S.; and n=118, Fred Hutchinson Cancer Research Center, U.S.) (ClinicalTrials.gov Identifier: NCT02328677) [34]. Clinical, demographic, and lifestyle-related characteristics were collected for all participants and harmonized across all cohorts within the FOCUS consortium. All studies were approved by local medical ethics committees and the current study was performed in accordance with the Declaration of Helsinki. All

participants had histologically confirmed stage I-III colorectal cancer and provided written informed consent. Brief details on cohorts and data collection can be found in the

Supplementary Methods.

Biochemical analysis

Plasma or serum samples were collected at colorectal cancer diagnosis, shipped on dry ice for analysis at the laboratory of BEVITAL AS (Bergen, Norway; www.bevital.no). Plasma samples were used for COLON (EDTA), EnCoRe (EDTA) and CORSA (EDTA and heparin), and serum samples were used for the ColoCare sites. Folate concentrations were measured as the sum of the folate species 5-methyl-tetrahydrofolate (5-mTHF) and 4-alpha-hydroxy-5-methyl-tetrahydrofolate (hmTHF) [35]. In addition, we assessed the concentration of unmetabolized folic acid and folate catabolites, p-aminobenzoylglutamate (pABG) and p-acetamidobenzoylglutamate (apABG) [36]. Concentrations of 5-mTHF, hmTHF, folic acid, pABG, and apABG were quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS) [36]. Details on sample preparation are provided in the **Supplementary Methods**.

Study end points

Study end points included recurrence, disease-free and overall survival. Recurrence was defined as locoregional or distant recurrence after complete tumor resection. Overall survival events were investigated by using death from any cause in the analysis. Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis. For all outcomes, follow-up time was calculated starting from the date of blood collection. Further details are provided in the **Supplementary Methods**.

Statistical analysis

Multivariable Cox proportional hazard models were used to investigate the associations of circulating concentrations of folate, folic acid, and folate catabolites with recurrence, overall and disease-free survival. The proportional hazard assumption for Cox proportional hazard models was assessed by using Schoenfeld residuals; with no evidence of non-proportionality being detected. Folate concentrations were analyzed continuously (log2 transformed) as well as in tertiles in which the lowest tertile was used as the reference. Tertiles were defined based upon the total study population. *P*-trend values were computed for folate concentration tertiles using the medians of the corresponding tertiles. Folic acid,

pABG, and apABG were investigated in two ways. First, concentrations were categorized into a dichotomous variable to compare patients with a detectable concentration to patients with concentration equal to or below the level of detection (LOD). Second, for patients with detectable concentrations, we also performed analyses similar to those for folate. The associations of tertiles of circulating concentrations of folate, folic acid, pABG and apABG with recurrence, overall and disease-free survival were also investigated using cohort-specific tertiles. The tertile values of the cohort-specific tertiles were used continuously to compute the p-value for linear trend.

Crude hazard ratios (HRs) and HRs adjusted for age at diagnosis, sex, cohort, and chemotherapy status (receiving no chemotherapy, only neo-adjuvant chemotherapy, only adjuvant chemotherapy, or both neo-adjuvant and adjuvant chemotherapy) were calculated for folates. Log2 transformed creatinine concentrations were additionally added as a covariate for pABG and apABG analyses, because these have high renal clearance, and it is thus important to take kidney function into account [37]. Adjustment for other known potential confounding factors, i.e. disease stage, body mass index [38], alcohol intake [39], smoking [40], analytical plate and batch, did not markedly influence the estimates (<10%) and were therefore not included in the final model.

Subgroup analyses were conducted and presented by forest plots to assess potential effect measure modification by cohort, disease stage, tumor location, sex, neo- and/or adjuvant treatment, and dietary supplement use.

Cox proportional hazard models were computed in SAS version 9.4 software (Cary, NC) using two-sided tests. Forest plots, including heterogeneity tests using the package '*metafor*' [41], were prepared in R, version 3.3.6. A *p*-value of < 0.05 was considered statistically significant. Sensitivity analyses and further details concerning statistical methods are described in the **Supplementary Methods**.

Results

Study population

Baseline characteristics of the total study population (n=2024) and by cohort are displayed in **Table 1**. The majority of the participants were men (64%), overweight (43%) and former or never smokers (53% and 34%). The median (interquartile range (IQR)) age was

66 (60-73) years, and 27%, 30%, and 41% of the participants presented with stage I, II, and III, respectively. Colon cancer was diagnosed among 62% of participants while 38% had rectal cancer. Dietary supplement use was reported by 41% of the total study population and 20% of the participants reported to use dietary supplements containing folic acid.

The median (IQR) circulating concentration of folate, i.e. the sum of 5-mTHF and hmTHF, was 15.0 (9.8-24.5) nmol/l. Among participants with a detectable folic acid (n=301), pABG (n=1946), and apABG (n=1801) concentration, median (IQR) concentrations were 1.0 (0.7-1.9) nmol/l, 2.5 (1.0-5.4) nmol/l, and 0.7 (0.5-1.0) nmol/l, respectively. There was a substantial difference in folate and folic acid concentrations between cohorts; the U.S. cohorts, i.e. ColoCare HCI and ColoCare FHCRC, showed higher concentrations compared to the European cohorts, i.e. COLON, EnCoRe, CORSA and ColoCare HD, which is consistent with folic acid fortification being present in the U.S. [16]. Concentrations of pABG were higher in COLON and EnCoRe when compared with CORSA and the ColoCare cohorts while ApABG showed comparable concentrations for all cohorts.

The median follow-up time for the study population was 3.7 years. During follow-up, 288 participants died from any cause and 258 participants experienced a recurrence. Recurrence cases consisted of locoregional (n=66) and distant recurrences (n=217); 21 participants had both a locoregional and distant recurrence. Recurrence prior to death was experienced by 113 of the 288 participants who died during the study.

Table 1 Baseline characteristics of the total study population and stratified by cohort

COHORTS						
Total population (n=2024)	COLON (n=1094)	EnCoRe (n=297)	CORSA (n=209)	ColoCare HD (n=260)	ColoCare HCI (n=46)	ColoCare FHCRC (n=118)
Men, n (%)	698 (63.8)	200 (67.3)	139 (66.5)	173 (66.5)	31 (67.4)	63 (53.4)
Age at diagnosis (years), median (IQR)	66.5 (61.4-72.1)	67.0 (61.0-73.0)	69.1 (60.0-75.6)	65.5 (56.5-73.0)	59.5 (52.0-68.0)	59.0 (49.0-68.0)
Body mass index (kg/m ²), median (IQR)	26.0 (23.9-28.7)	27.6 (24.9-30.7)	27.2 (24.7-30.1)	26.3 (23.8-29.1)	28.4 (25.1-32.3)	28.2 (23.7-33.1)
Underweight, <18.5, n (%)	10 (0.9)	1 (0.3)	1 (0.5)	2 (0.8)	0 (0.0)	3 (2.5)
Normal weight, 18.5-24.9, n (%)	419 (38.5)	75 (25.4)	52 (26.7)	90 (34.8)	10 (23.8)	38 (32.2)
Overweight, 25-29.9, n (%)	471 (43.3)	127 (43.1)	93 (47.7)	121 (46.7)	16 (38.1)	35 (29.7)
Obese, ≥30, n (%)	188 (17.3)	92 (31.2)	49 (25.1)	46 (17.8)	16 (38.1)	42 (35.6)
Unknown/missing, n	6	2	14	1	4	0
Smoking, n (%)						
Current	121 (11.4)	39 (13.4)	35 (17.3)	45 (18.8)	4 (9.5)	7 (7.7)
Former	630 (59.5)	160 (55.0)	74 (36.6)	114 (47.5)	10 (23.8)	40 (44.0)
Never	308 (29.1)	92 (31.6)	93 (46.0)	81 (33.8)	28 (66.7)	44 (48.4)
Unknown/missing, n	35	6	7	20	4	27
Stage of disease, n (%)						
I	280 (25.6)	86 (29.0)	78 (37.3)	64 (24.6)	7 (15.2)	28 (23.7)
II	330 (30.2)	59 (19.9)	52 (24.9)	104 (40.0)	13 (28.3)	41 (34.8)
III	463 (42.3)	142 (47.8)	52 (24.9)	92 (35.4)	26 (56.5)	49 (41.5)
Unspecified/unknown, n	21 (1.9)	10 (3.4)	27 (12.9)	0 (0.0)	0 (0.0)	0 (0.0)
Tumor location ¹ , n (%)						
Colon	720 (65.8)	179 (60.3)	127 (61.7)	136 (52.3)	26 (57.8)	64 (54.2)
Rectal	374 (34.2)	118 (39.7)	79 (38.3)	124 (47.7)	19 (42.2)	54 (45.8)
Unknown/missing, n	0	0	3	0	1	0
Neo-adjuvant treatment, n (%)						
Yes	253 (23.1)	81 (27.3)	17 (8.1)	63 (24.2)	15 (32.6)	29 (24.8)
No	840 (76.9)	216 (72.7)	192 (91.9)	197 (75.8)	31 (67.4)	88 (75.2)

Surgery, n (%)	Unknown/missing, n	2	1	0	0	0	1
	Yes	1963 (97.2)	1084 (99.3)	269 (90.9)	187 (89.9)	260 (100.0)	117 (99.1)
	No	57 (2.8)	8 (0.7)	27 (9.1)	21 (10.1)	0 (0.0)	1 (0.9)
Adjuvant treatment, n (%)	Unknown/missing, n	4	2	1	1	0	0
	Yes	596 (29.8)	254 (23.6)	97 (32.8)	59 (28.4)	95 (36.5)	62 (53.0)
	No	1406 (70.2)	823 (76.4)	199 (67.2)	149 (71.6)	165 (63.5)	55 (47.0)
Adherence to physical activity guidelines ² , n (%)	Unknown/missing, n	22	17	1	1	0	1
	Yes	1115 (65.7)	778 (73.7)	217 (74.6)	-	77 (33.3)	29 (38.2)
	No	581 (34.3)	278 (26.3)	74 (25.5)	-	154 (66.7)	47 (61.8)
Dietary supplement use ³ , n (%)	Unknown/missing, n	328	38	6	209	29	42
	Any	682 (40.5)	451 (42.6)	109 (36.7)	-	58 (24.5)	42 (89.4)
	Containing folic acid	342 (20.3)	269 (25.4)	60 (20.1)	-	4 (1.7)	8 (17.0)
Total folate concentration (nmol/l), median (IQR)	Unknown supplement use, n	341	34	0	209	23	71
	Any	15.0 (9.8-24.5)	12.5 (8.6-18.3)	13.8 (9.8-19.0)	17.5 (11.7-24.2)	22.0 (15.3-31.6)	47.9 (33.3-74.5)
Participants with detectable folic acid concentrations ⁴ , n (%)	Unknown supplement use, n	341	34	0	209	23	71
	Any	15.0 (9.8-24.5)	12.5 (8.6-18.3)	13.8 (9.8-19.0)	17.5 (11.7-24.2)	22.0 (15.3-31.6)	47.9 (33.3-74.5)
Detectable folic acid concentrations (nmol/l), median (IQR)	Unknown supplement use, n	341	34	0	209	23	71
	Any	15.0 (9.8-24.5)	12.5 (8.6-18.3)	13.8 (9.8-19.0)	17.5 (11.7-24.2)	22.0 (15.3-31.6)	47.9 (33.3-74.5)
Participants with detectable pABG concentrations ⁵ , n (%)	Unknown supplement use, n	341	34	0	209	23	71
	Any	15.0 (9.8-24.5)	12.5 (8.6-18.3)	13.8 (9.8-19.0)	17.5 (11.7-24.2)	22.0 (15.3-31.6)	47.9 (33.3-74.5)
Detectable pABG concentrations (nmol/l), median (IQR)	Unknown supplement use, n	341	34	0	209	23	71
	Any	15.0 (9.8-24.5)	12.5 (8.6-18.3)	13.8 (9.8-19.0)	17.5 (11.7-24.2)	22.0 (15.3-31.6)	47.9 (33.3-74.5)
Participants with detectable apABG concentrations ⁶ , n (%)	Unknown supplement use, n	341	34	0	209	23	71
	Any	15.0 (9.8-24.5)	12.5 (8.6-18.3)	13.8 (9.8-19.0)	17.5 (11.7-24.2)	22.0 (15.3-31.6)	47.9 (33.3-74.5)
Detectable apABG concentrations (nmol/l), median (IQR)	Unknown supplement use, n	341	34	0	209	23	71
	Any	15.0 (9.8-24.5)	12.5 (8.6-18.3)	13.8 (9.8-19.0)	17.5 (11.7-24.2)	22.0 (15.3-31.6)	47.9 (33.3-74.5)
Total energy intake ⁷ (kcal/day), median (IQR)	Unknown supplement use, n	341	34	0	209	23	71
	Any	15.0 (9.8-24.5)	12.5 (8.6-18.3)	13.8 (9.8-19.0)	17.5 (11.7-24.2)	22.0 (15.3-31.6)	47.9 (33.3-74.5)

Unknown/missing, n	545	48	12	209	180	32	64
Alcohol intake ⁷ (g/day), median (IQR)	8.1 (0.9-20.9)	8.5 (1.0-20.7)	7.0 (0.6-20.6)	-	10.4 (3.1-27.6)	-	-
Unknown/missing, n	393	48	12	209	28	32	64
Follow-up time ⁸ , median (range)	3.7y (4d-15.4y)	4.1y (25d-8.4y)	2.9y (23d-5.8y)	5.7y (23d-15.4y)	2.1y (4d-5.5y)	2.2y (37d-3.7y)	5.1y (0.3y-10.1y)
Deceased ⁹ , n (%)	288 (14.2)	138 (12.6)	28 (9.4)	66 (31.6)	28 (10.8)	4 (8.7)	24 (20.3)
Recurrence ¹⁰ , n (%)							
Yes	258 (13.0)	144 (13.2)	31 (10.4)	26 (12.4)	30 (13.3)	6 (14.6)	21 (18.1)
Unknown/missing, n	46	4	0	0	35	5	2
Location of the recurrence, n (%)							
Locoregional	66 (3.3)	41 (3.8)	7 (2.4)	7 (3.4)	8 (3.6)	0 (0.0)	3 (2.6)
Distant	214 (10.6)	128 (11.7)	26 (8.8)	21 (10.1)	18 (8.0)	5 (12.2)	16 (13.8)
Unknown location	7 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	4 (1.8)	1 (2.4)	2 (1.7)
Disease-free survival ¹¹ , n (%)							
Participants with at least one event	428 (21.7)	215 (19.7)	49 (16.5)	77 (36.8)	49 (21.8)	7 (17.1)	31 (26.7)

¹¹Tumor location is defined as colon (cecum, appendix and ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon) and rectal (rectosigmoid junction and rectum) cancer.

²At least 150 minutes per week of moderate-to-vigorous physical activity.

³Dietary supplement use is defined as the use of single micronutrients and/or the use of multivitamins in the last year for COLOn and EnCoRe and in the last month for the ColoCare sites. Dietary supplement use was not collected in the CORSA study.

⁴Detectable folic acid concentrations > 0.52 nmol/l.

⁵Detectable pABG concentrations > 0.08 nmol/l.

⁶Detectable apABG concentrations > 0.13 nmol/l.

⁷Total energy and alcohol intake at diagnosis; data not shown for cohorts in which > 50% of participants had missing FFQ data.

⁸Follow-up time calculated using overall survival.

⁹Overall survival events were investigated by using death from any cause in the analysis.

¹⁰Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection; n=29 have a locoregional and distant recurrence simultaneously, n=46 with missing/incomplete recurrence data.

¹¹Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis, n=112 of the 288 deceased patients experienced a recurrence prior to death.

Baseline characteristics by tertiles of folate and comparing participants with (n=301) and without (n=1723) detectable folic acid concentrations and by tertiles of folic acid concentrations are described in **Supplementary Tables 1 and 2**, respectively. As expected, the most dietary supplement use was reported in the highest folate and folic acid tertile. Baseline characteristics by tertiles of pABG and apABG concentrations can be found in **Supplementary Tables S3**, respectively.

Folates and study end points

Associations between circulating concentrations of folate, folic acid, pABG and apABG with recurrence, overall and disease-free survival are described in **Table 2**. No statistically significant associations were observed for folate, pABG, and apABG, with HRs ranging from 0.92 to 1.16. A moderate non-significant linear trend (p -value=0.11) for improved overall survival, but not for recurrence and disease-free survival, was observed with increasing tertiles of folate concentrations. Similar findings for recurrence, overall and disease-free survival were observed when using cohort-specific tertiles of folate concentrations (**Supplementary Table S4**). Increasing tertiles of apABG concentrations showed a non-significant linear trend towards a greater risk of death (HR_{T2vsT1} (95% confidence interval (95% CI)): 1.25 (0.91 to 1.70) and HR_{T3vsT1}: 1.31 (0.93 to 1.83), p for trend= 0.15), but not for recurrence and disease-free survival. Using cohort-specific tertiles of apABG concentrations, similar trends and effect estimates were observed, but the p -value for linear trend for overall survival became statistically significant (p for trend = 0.03; **Supplementary Table S4**).

Comparing patients with detectable folic acid concentrations to patients without detectable folic acid for any outcome showed no statistically significant associations (**Table 2**). However, among patients with detectable folic acid concentrations, more recurrences were observed (HR (95% CI) of 1.31 (1.02 to 1.58) for each two-fold increase in folic acid concentration). A statistically significant, linear trend (p -value: 0.03) was observed across folic acid tertiles. Tertile 2 and 3 showed a non-statistically significant increased risk of death compared to the lowest tertile of folic acid concentrations (HR_{T2vsT1} (95% CI): 1.26 (0.56 to 3.61) and HR_{T3vsT1}: 1.57 (0.70 to 3.55)). A borderline, non-statistically significant, linear trend (p -value: 0.09) was observed across folic acid tertiles for disease-free survival, HR_{T2vsT1} (95% CI): 1.58 (0.82 to 3.04) and HR_{T3vsT1}: 2.01 (1.01 to 3.97) (**Table 2**). Analyses using

cohort-specific tertiles of folic acid concentrations showed similar effect estimates and linear trends for recurrence, overall and disease-free survival (**Supplementary Table S4**).

Sensitivity analyses excluding participants who died or experienced a recurrence within the first 100 days after diagnosis or excluding patients who did not receive surgery or with unknown surgery status and limiting analysis to recurrence events within the first two after diagnosis showed similar trends to the overall analysis (*data not shown*). Sensitivity analyses excluding participants from whom blood was collected during or after any type of treatment (n=275, 14%) showed comparable linear trends compared with the main analyses (**Supplementary Table S5**).

Table 2 Associations between circulating concentrations of folate, folic acid, pABG, and apABG and recurrence and survival

Recurrence ¹											Death from any cause (overall survival) ²			Recurrence or death from any cause (disease-free survival) ³					
Median (IQR)			No. of events/at risk	Crude HR (95% CI)	Adj. HR (95% CI) ⁴	No. of deaths/at risk			Crude HR (95% CI)	Adj. HR (95% CI) ⁴	No. of events/at risk			Crude HR (95% CI)	Adj. HR (95% CI) ⁴				
Folate (nmol/l)																			
Continuous ⁵											258/1973	1.06 (0.94 to 1.20)	1.05 (0.91 to 1.21)	288/2024	0.99 (0.88 to 1.11)	0.92 (0.80 to 1.05)	428/1973	1.01 (0.92 to 1.11)	0.98 (0.88 to 1.10)
T1 ⁶											84/672	1.00 (referent)	1.00 (referent)	98/675	1.00 (referent)	1.00 (referent)	138/672	1.00 (referent)	1.00 (referent)
T2 ⁶											83/659	1.02 (0.75 to 1.38)	1.01 (0.74 to 1.37)	100/675	1.01 (0.76 to 1.34)	0.94 (0.70 to 1.24)	150/659	1.09 (0.86 to 1.37)	1.02 (0.81 to 1.30)
T3 ⁶											91/642	1.14 (0.85 to 1.54)	1.14 (0.81 to 1.60)	90/674	0.91 (0.68 to 1.21)	0.77 (0.56 to 1.07)	140/642	1.02 (0.80 to 1.29)	0.94 (0.72 to 1.23)
P _{trend}												0.35	0.40		0.45	0.11		0.98	0.58
Folic acid (nmol/l)																			
Undetectable ⁷											216/1677	1.00 (referent)	1.00 (referent)	237/1723	1.00 (referent)	1.00 (referent)	354/1677	1.00 (referent)	1.00 (referent)
Detectable ⁷											42/296	1.10 (0.80 to 1.54)	1.18 (0.84 to 1.66)	51/301	1.16 (0.85 to 1.57)	1.14 (0.83 to 1.56)	74/296	1.15 (0.89 to 1.47)	1.19 (0.92 to 1.54)
Continuous ⁸											42/296	1.23 (1.05 to 1.44)	1.31 (1.02 to 1.58)	51/301	1.21 (1.03 to 1.42)	1.12 (0.94 to 1.34)	74/296	1.16 (1.01 to 1.33)	1.13 (0.97 to 1.32)
T1 ⁹											8/98	1.00 (referent)	1.00 (referent)	10/100	1.00 (referent)	1.00 (referent)	15/98	1.00 (referent)	1.00 (referent)
T2 ⁹											14/99	1.78 (0.75 to 4.24)	1.86 (0.76 to 4.56)	18/101	1.67 (0.77 to 3.61)	1.26 (0.56 to 2.84)	27/99	1.69 (0.90 to 3.18)	1.58 (0.82 to 3.04)
T3 ⁹											20/99	2.59 (1.14 to 5.89)	3.12 (1.22 to 8.00)	23/100	2.20 (1.05 to 4.63)	1.57 (0.70 to 3.55)	32/99	2.15 (1.16 to 3.98)	2.01 (1.01 to 3.97)
P _{trend}												0.03	0.03		0.06	0.29		0.03	0.09
pABG (nmol/l)																			
Undetectable											9/69	1.00 (referent)	1.00 (referent)	13/78	1.00 (referent)	1.00 (referent)	18/69	1.00 (referent)	1.00 (referent)
Detectable ⁷											249/1904	0.99 (0.51 to 1.92)	1.06 (0.63 to 2.14)	275/1946	0.69 (0.40 to 1.21)	0.90 (0.50 to 1.63)	410/1904	0.78 (0.49 to 1.26)	0.98 (0.60 to 1.62)
Continuous ⁸											249/1904	1.01 (0.94 to 1.09)	1.03 (0.94 to 1.13)	275/1946	0.92 (0.86 to 0.99)	1.01 (0.93 to 1.11)	410/1904	0.95 (0.89 to 1.01)	0.99 (0.92 to 1.06)
T1 ⁹											86/634	1.00 (referent)	1.00 (referent)	104/647	1.00 (referent)	1.00 (referent)	159/634	1.00 (referent)	1.00 (referent)
T2 ⁹											74/635	0.89 (0.65 to 1.21)	0.95 (0.67 to 1.33)	86/649	0.91 (0.68 to 1.21)	1.16 (0.84 to 1.59)	122/635	0.85 (0.67 to 1.08)	0.95 (0.74 to 1.24)
T3 ⁹											89/635	1.00 (0.74 to 1.34)	1.03 (0.72 to 1.47)	85/650	0.74 (0.55 to 0.98)	1.08 (0.76 to 1.53)	129/635	0.80 (0.63 to 1.01)	0.94 (0.71 to 1.25)
P _{trend}												0.83	0.73		0.04	0.90		0.09	0.71
apABG (nmol/l)																			
Undetectable											26/215	1.00 (referent)	1.00 (referent)	27/223	1.00 (referent)	1.00 (referent)	41/215	1.00 (referent)	1.00 (referent)

Detectable ⁷	0.7 (0.5 to 1.0)	232/1758	1.19 (0.79 to 1.78)	1.24 (0.82 to 1.88)	261/1801	1.33 (0.90 to 1.98)	1.11 (0.94 to 1.31)	387/1758	1.26 (0.92 to 1.75)	1.20 (0.87 to 1.67)
Continuous ⁸	0.7 (0.5 to 1.0)	232/1758	1.04 (0.89 to 1.22)	1.02 (0.85 to 1.22)	261/1801	1.28 (1.11 to 1.47)	1.16 (0.99 to 1.36)	387/1758	1.15 (1.02 to 1.30)	1.05 (0.91 to 1.20)
T1 ⁹	0.4 (0.4 to 0.5)	73/587	1.00 (referent)	1.00 (referent)	79/600	1.00 (referent)	1.00 (referent)	120/587	1.00 (referent)	1.00 (referent)
T2 ⁹	0.7 (0.7 to 0.8)	86/585	1.25 (0.91 to 1.71)	1.23 (0.90 to 1.70)	89/600	1.35 (1.00 to 1.83)	1.25 (0.91 to 1.70)	135/585	1.27 (0.99 to 1.62)	1.21 (0.94 to 1.56)
T3 ⁹	1.2 (1.0 to 1.5)	73/586	1.12 (0.81 to 1.55)	1.08 (0.76 to 1.55)	93/601	1.61 (1.19 to 2.19)	1.31 (0.93 to 1.83)	132/586	1.39 (1.08 to 1.78)	1.21 (0.92 to 1.59)
P _{trend}			0.60	0.80		0.002	0.15		0.01	0.23

¹Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection. A HR > 1.00 should be interpreted as an increased risk of recurrence.
²Overall survival events were investigated by using death from any cause in the analysis. A HR > 1.00 should be interpreted as a reduced overall survival (more deaths).
³Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis. A HR > 1.00 should be interpreted as a reduced disease-free survival (more deaths and/or recurrences).
⁴Adjusted for age, sex, chemotherapy status, and cohort for folate and folic acid. pABG and apABG were additionally adjusted for log2 transformed creatinine concentrations. Stage was tested as potential confounder in all models, but it did not influence the effect estimates.
⁵Analysis performed using log2 transformed concentrations. Thus, HRs represent a doubling in folate concentrations.
⁶Tertile cut-off values for folate were 11.5 nmol/l and 20.1 nmol/l; analysis performed using non-log2 transformed concentrations.
⁷Detectable concentrations > 0.52 nmol/l for folic acid, > 0.08 nmol/l for pABG, and > 0.13 nmol/l for apABG; analysis performed using log2 transformed concentrations.
⁸Analysis only performed in the participants with a detectable value; analysis performed using log2 transformed concentrations. HRs thus represent a doubling in concentrations.
⁹Analysis only performed for participants with detectable folic acid, pABG, or apABG. Tertile cut-off values were 0.75 nmol/l and 1.46 nmol/l for folic acid, 1.39 nmol/l and 4.18 nmol/l for pABG, and 0.59 nmol/l and 0.89 nmol/l for apABG; analysis performed using non-log2 transformed concentrations.
¹⁰LOD: level of detection.

Subgroup analyses by clinical and lifestyle-related factors

Subgroup analyses for folate concentrations are shown in **Figure 1**. No statistically significant associations were observed for any outcome when evaluated within individual cohorts, likely attributable to small sample sizes. Stage I patients had a statistically significantly lower risk of recurrence with higher folate concentrations (HR (95%CI): 0.34 (0.20 to 0.58)) while this association was not observed in stage II-III patients ($I^2 = 89.4\%$, $p = 0.0003$).

Supplementary Figure S3 illustrates cohort-specific tertiles of folate concentrations in relation to recurrence, overall and disease-free survival by individual cohort. Overall, there was no sign of a dose-response relationship within cohorts for recurrence, overall and disease-free survival.

Subgroup analyses for folic acid concentrations are shown in **Figure 2**. Patients with detectable folic acid concentrations who received neo-adjuvant treatment ($n=63$) had significantly higher risk of recurrence with higher folic acid concentrations (HR: 2.03 (1.41 to 2.92)), which was not observed in patients who did not receive neo-adjuvant treatment ($I^2 = 61.1\%$, $p = 0.08$).

Subgroup analyses for pABG and apABG are reported in **Supplementary Figure S2** and **Supplementary Figure S3**, respectively.

Discussion

Principal findings

We investigated circulating concentrations of folate, folic acid, and folate catabolites pABG and apABG, in relation to recurrence and survival among patients diagnosed with stage I-III colorectal cancer within the FOCUS international consortium. No statistically significant associations were observed for folate, pABG, and apABG concentrations. In contrast, more colorectal cancer recurrences were observed among patients with higher compared to lower circulating folic acid concentrations.

Folate

Ranges of folate concentration observed in our study population were comparable to

prior studies with colorectal cancer patients measuring folate at diagnosis, despite the different analytical methods used to quantify folate [29, 30]. Our results, showing no association between folate concentrations and recurrence and survival, are consistent with the only other, smaller, study in stage I-III CRC patients [29]. However, when we investigated subgroups in our large consortium, a statistically significant lower risk of recurrence was observed with higher compared to lower folate concentrations in stage I patients, and not in stage II-III patients. This intriguing result has not been reported before and requires further investigation.

Folic acid

Unmetabolized folic acid was only detected in the circulation of 15% of included participants which could be explained by the fact that folic acid is only detectable in case of recent and excessive intakes [28] or due to low DHFR activity. As was hypothesized, higher concentrations of folic acid were associated with increased colorectal cancer recurrence in our study population. This suggests that folic acid may facilitate growth of potentially remaining tumor cells in the body similar as what is hypothesized concerning premalignant lesions during colorectal cancer development [2, 27]. Furthermore, higher concentrations of folic acid were associated with reduced disease-free survival and higher concentration of folic acid may also be associated with a higher risk of death. However, the association with overall survival was not statistically significant, which could potentially be explained by low sample size. Therefore, this study highlights the need for more research regarding excessive intakes, through high-dose folic acid supplement use or consumption of fortified foods in relation to cancer prognosis.

Figure 1 Forest plots of subgroup analyses reporting hazard ratios and corresponding 95% CI for a doubling in folate concentrations and recurrence, overall and disease to free survival. Weights of the effect estimates are from random effects meta to analysis; square dots represent the hazard ratio of each subgroup while the diamonds represent the hazard ratio of all subgroups combined. Heterogeneity among subgroups was evaluated using the I^2 index. CI: confidence interval; IQR: interquartile range; Q: Heterogeneity Cochran's Q test; df: degrees of freedom; p: p -value; I^2 : Heterogeneity I^2 statistic.

Figure 2 Forest plots of subgroup analyses reporting hazard ratios and corresponding 95% CI for a doubling in folic acid concentration s and recurrence, overall and disease to free survival. Weights of the effect estimates are from random effects meta to analysis; square dots represent the hazard ratio of each subgroup while the diamonds represent the hazard ratio of all subgroups combined. Heterogeneity among subgroups was evaluated using the I^2 index. CI: confidence interval; IQR: interquartile range; Q: Heterogeneity Cochran's Q test; df: degrees of freedom; p: p -value; I^2 : Heterogeneity I^2 statistic. Note: No. of recurrence and disease to free events for ColoCare HD, ColoCare HCl and stage I participants were too limited to meet the Cox proportional hazard model convergence criterion and are therefore not presented in the forest plot.

Folate catabolites

This is the first study investigating the association between folate catabolites and colorectal cancer recurrence and survival. No associations between folate catabolites and colorectal cancer recurrence and survival were observed. However, a moderate trend towards more deaths with increasing apABG concentrations, the main folate catabolite, was observed, which warrants replication. Folate catabolism plays a role in regulating folate homeostasis and catabolites are suggested to be potential functional markers of folate status [21]. Increased folate catabolism has been reported earlier in colon tumor cells [20] and, recently, increased circulating concentrations of folate catabolites are hypothesized to be a result of increased inflammation [42]. The role of folate catabolites in colorectal cancer recurrence and survival remains to be elucidated.

Strengths and limitations

This is the first large multi-cohort consortium investigating concentrations of folate, folic acid, and folate catabolites in relation to colorectal cancer recurrence and survival, including >2000 stage I-III colorectal cancer patients. Quantifying folate using LC-MS/MS allowed us to discern separate folate species, including folate catabolites, and is therefore considered a more powerful method to measure folate status [28, 36] compared to the microbiological assay used in previous studies [29, 30]. Patients in this study population diagnosed with stage I-III colorectal cancer were included from several European and U.S. cohort studies, which enabled us to explore a wide range of folate, folic acid and catabolites concentrations. Because of the large sample size, we were also able to conduct subgroup analyses. Although our sample size was large, we acknowledge that a relatively small proportion of participants originated from U.S. cohorts (n=188), which did not allow us to comprehensively compare European and U.S. cohorts. Median follow-up time was relatively short, with 3.7 years, although it should be noted that most recurrences tend to occur in the first 2-3 years after colorectal cancer diagnosis [43-45] and we had a broad range with a maximum follow-up time of 15.4 years. In addition, concentrations of folate species were only assessed in a single sample, which is a further limitation. A single sample may not capture past exposures and exposures after cancer diagnosis related to lifestyle factors or daily variability in metabolites [44]. Lastly, colorectal cancer-specific survival could not be investigated because cause of death was not available for all cohorts.

Future research

Future research investigating colorectal cancer prognosis should include unmetabolized folic acid and folate catabolites. The folate catabolites pABG and apABG have not been extensively studied in the context of colorectal cancer, while they might provide a broader understanding of folate kinetics in colorectal cancer patients. Furthermore, the importance of unmetabolized folic acid, and the potential different biochemical effect compared to natural folate, in relation to human health is still unclear [28, 46] while dietary supplement use is common among cancer patients, and food fortification is applied in 81 countries [47]. Our current findings provide relevant leads for future studies on the underlying mechanisms. A potentially interesting mechanism might be through immune function, as increased unmetabolized folic acid concentrations have also been linked to reduced immune function in the past [48]. Furthermore, it might be worthwhile to consider variations in genes related to FOCM, such as MTHFR and DHFR [49] as well as potential genetic predisposition for colorectal cancer recurrence and survival [45, 50, 51] when investigating whether associations with recurrence and survival potentially vary between persons with a different genetic background.

Conclusion

We reported that folate and folate catabolites concentrations in our international prospective cohort study population were not associated with colorectal cancer recurrence and survival. We observed that higher folic acid concentrations are associated with an increased risk of colorectal cancer recurrence in stage I-III colorectal cancer patients. A better understanding of the potential harmful effects of unmetabolized folic acid in the circulation, specifically among colorectal cancer patients, is warranted. Although dietary intake of folic acid through fortified foods or dietary supplements was not assessed in the current study, awareness may be required concerning the potential risk of excessive intakes, particularly among patients diagnosed with colorectal cancer.

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Supplementary materials

Supplementary Methods

Study population

The FOCUS consortium was initiated to investigate biomarkers related to Folate-dependent One-carbon metabolism in colorectal Cancer recurrence and Survival. The six prospective cohort studies included in this consortium have been described before. Briefly, the COLON [1] and EnCoRe [2] studies are ongoing prospective cohort studies that recruit newly diagnosed colorectal cancer patients since 2010 and 2012, respectively. Participants were recruited at time of diagnosis from eleven hospitals in the Netherlands for the COLON study and from three hospitals in the Netherlands for the EnCoRe study. CORSA is an ongoing study since 2003, recruiting colorectal cancer patients in cooperation with the province-wide screening project “Burgenland Prevention Trial of Colorectal Disease with Immunological Testing” (B-PREDICT) using fecal occult blood testing (FOBT). FOBT-positive tested individuals subsequently received a complete colonoscopy. Additional colorectal cancer patients were recruited at diagnosis at four hospitals in Vienna. ColoCare is an ongoing, international, prospective cohort study [3]. Patients were recruited since 2010 at the University Hospital of Heidelberg and the National Center for Tumor Diseases in Heidelberg, Germany (ColoCare HD). ColoCare patients were also recruited since 2015 at Huntsman Cancer Institute (Salt Lake City, U.S.)(ColoCare HCI) and since 2007 at Fred Hutchinson Cancer Research Center (Seattle, U.S.)(ColoCare FHCRC).

Data collection

Demographic and lifestyle characteristics including age at colorectal cancer diagnosis, sex, weight, height, smoking history, and dietary supplement use were collected through cohort-specific self-administered questionnaires (COLON, EnCoRe, CORSA and ColoCare) and home visits (EnCoRe). Total energy intake (kcal/day) and alcohol use (kcal/day) were assessed at diagnosis using standardized Food Frequency Questionnaires (FFQ) in COLON, EnCoRe and the ColoCare cohorts. Physical activity was assessed using the validated Short QuesTionnaire to ASsess Health enhancing physical activity (SQUASH) in COLON and EnCoRe [1, 2]. For ColoCare, questions regarding physical activity were included in cohort-specific questionnaires [3]. Participants included from CORSA did not have FFQ data, intake of dietary supplements, nor physical activity available as these variables are not routinely collected in this cohort.

Clinical data, including colorectal cancer stage, tumor site, resection status, and treatment regimen were obtained from medical records. Clinical outcome data, including vital status and disease recurrence, were abstracted from medical records and through linkage with national cancer registries.

Sample preparation

Blood samples were collected shortly after colorectal cancer diagnosis and mostly before neo-adjuvant therapy or surgery (87%). Samples were sent and measured in two batches; the first batch was measured between November 2016 and March 2017. The second batch was analyzed between November 2017 and March 2018, to allow for longer follow-up time of participants and therefore more samples to analyze. Samples were analyzed in 96-well plates; each plate consisted of 86 study samples supplemented by one blank sample (water), two different in-house quality control samples in duplicate and five calibrator samples.

In addition to BEVITAL in-house quality control samples, duplicates of COLON samples (n=97) were included in both batches. The mean value for duplicate samples was calculated for concentrations included in the current study. Regression coefficients for COLON duplicates were 0.94, 0.92, 0.92, 0.90 and 0.71 for 5-mTHF, hmTHF, folic acid, pABG, and apABG, respectively.

Blood samples from the ColoCare cohorts were 1:2 diluted for analysis due to limited sample volume availability. The dilution factor was considered in concentrations by BEVITAL prior to providing biochemical analysis data.

Study end points

Recurrence was defined as locoregional (including local recurrence, in either the tumor bed or bowel wall, and affected regional lymph nodes) or distant (metastasis) recurrence after complete tumor resection. If tumor resection status was incomplete, newly diagnosed colorectal tumors were defined as a progression of the initial colorectal cancer diagnosis and were therefore not classified as a disease recurrence in the present study (n=10). Patients with a missing or unknown recurrence status (n=46) were excluded from recurrence and disease-free survival analyses. Follow-up time for recurrence was calculated as the time between the date of blood collection and the date of recurrence. Date of colorectal cancer recurrence was defined as the date when recurrence status was updated or the last known date for follow-up (for example due to death or immigration), whichever came first. The date of recurrence was defined as the date of confirmatory imaging, or, in cases that needed tissue diagnosis confirmation, the date of biopsy.

The definition of overall survival was based on using death from any cause in the analysis. Follow-up time was calculated as the time between the date of blood collection to the date of death, the last date vital status was updated, or the date of end of follow-up (for example due to immigration), whichever came first.

Disease-free survival was defined by considering a recurrence or death from any cause as events in the analysis. Follow-up time was calculated as the time between the date of blood collection to the date of recurrence or death, whichever came first, or until the last date vital status was updated, the last date recurrence status was updated, or the date of end of follow-up (for example due to immigration), whichever came first.

For the cohorts COLON and EnCoRe, recurrence data was collected by the Netherlands Comprehensive Cancer Organization and survival data (date of death) were collected through linkage with the Municipal Personal Records Database. CORSA obtained recurrence data using the medical records in hospitals and survival data (date of death) using medical records and by checking with the Health Insurance Association. Lastly, the ColoCare sites collected data on recurrence using medical records and by sending questionnaires to the involved external physicians and oncologists. Date of death was obtained by the tumor registry of the National Center for Tumor Diseases (Heidelberg, ColoCare HD), by reviewing medical records, death registries, searching obituaries, and routine follow-up mailings (all ColoCare sites).

Statistical analysis

Clinical, demographic, and lifestyle characteristics were summarized for the total study population and by cohort using descriptive analysis. Hazard ratio (HR) estimates of Cox proportional hazard models for log₂-transformed continuous variables may be interpreted as the risk associated with a 2-fold increase in concentration of circulating folate, pABG, apABG, or folic acid.

Subgroup analyses presented using forest plots were conducted to assess potential effect measure modification by cohort (COLON/EnCoRe/CORSA/ColoCare HD/ColoCare HCI/ColoCare FHCRC), disease stage (stage I/stage II/stage III, excluding unknowns (n=58)), tumor location (colon/rectal, excluding unknowns (n= 4)), sex, neo- and/or adjuvant treatment (yes/no, excluding unknowns (n=16)), and dietary supplement use (any/containing folic acid/none, excluding unknowns (n=341)). Heterogeneity was explored with subgroup-specific risk estimates using a random-effects meta-analysis approach [4] and was evaluated using the I^2 index [5, 6]. In addition to the main analysis, dose-response plots for folate concentrations by cohort were

conducted using study-specific tertiles of folate concentrations. This was done because folic acid fortification is present in the U.S. and using tertiles based on the total study population for dose-response curves per cohort would thus not give a representative presentation.

Sensitivity analyses were performed by 1) excluding participants from whom blood was collected during or after any type of treatment, i.e. (neo-) adjuvant radio- or chemotherapy and/or surgery (n=275), by 2) excluding participants who died or experienced a recurrence within the first 100 days after diagnosis (n=33), by 3) excluding patients who did not receive surgery (n=57) or with unknown surgery status (n=4), and by 4) limiting analysis to recurrence events within the first two years after diagnosis (n=188 recurrence events).

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Supplementary Table S1 Baseline characteristics stratified by tertiles of folate

	Folate tertiles		
	Tertile 1 (n=675)	Tertile 2 (n=675)	Tertile 3 (n=674)
Men, n (%)	466 (69.0)	443 (65.6)	395 (58.6)
Age at diagnosis (years), median (IQR)	66.8 (61.2-73.0)	66.2 (61.0-72.9)	65.2 (58.0-72.1)
Body mass index (kg/m ²), median (IQR)	26.6 (24.2-29.4)	26.2 (24.0-29.0)	26.6 (24.1-30.0)
Underweight, <18.5, n (%)	3 (0.5)	7 (1.1)	7 (1.1)
Normal weight, 18.5-24.9, n (%)	224 (33.6)	241 (36.1)	219 (33.0)
Overweight, 25-29.9, n (%)	283 (42.5)	306 (45.8)	274 (41.3)
Obese, ≥30, n (%)	156 (23.4)	114 (17.1)	163 (24.6)
Unknown/missing, n	9	7	11
Smoking, n (%)			
Current	94 (14.5)	87 (13.4)	70 (11.2)
Former	354 (54.7)	365 (56.1)	309 (49.3)
Never	199 (30.8)	199 (30.6)	248 (39.6)
Unknown/missing	28	24	47
Stage of disease, n (%)			
I	178 (26.4)	177 (26.2)	188 (27.9)
II	190 (28.2)	190 (28.2)	219 (32.5)
III	286 (42.4)	290 (43.0)	248 (36.8)
Unspecified/unknown, n	21 (3.1)	18 (2.7)	19 (2.8)
Tumor location ¹ , n (%)			
Colon	436 (77.3)	411 (60.9)	405 (60.3)
Rectal	237 (35.2)	264 (39.1)	267 (39.7)
Unknown/missing, n	2	0	2
Neo-adjuvant treatment, n (%)			
Yes	153 (22.7)	163 (24.2)	142 (21.1)
No	521 (77.3)	512 (75.9)	531 (78.9)
Unknown/missing, n	1	0	1
Surgery, n (%)			
Yes	650 (96.4)	658 (97.8)	655 (97.3)
No	24 (3.6)	15 (2.2)	18 (2.7)
Unknown/missing, n	1	2	1
Adjuvant treatment, n (%)			
Yes	175 (26.2)	207 (31.0)	214 (32.1)
No	493 (73.8)	461 (69.0)	452 (67.9)
Unknown/missing, n	7	7	8
Cohort, n (%)			
COLON	487 (72.2)	384 (56.9)	223 (33.1)
EnCoRe	111 (15.4)	119 (17.6)	67 (9.9)
CORSA	51 (7.6)	76 (11.3)	82 (12.2)
ColoCare HD	25 (3.7)	85 (12.6)	150 (22.3)
ColoCare HCI	0 (0.0)	1 (0.2)	45 (6.7)
ColoCare FHCRC	1 (0.2)	10 (1.5)	107 (15.9)
Adherence to physical activity guidelines ² , n (%)			
Yes	413 (69.5)	400 (70.1)	302 (56.9)

	No	181 (30.5)	171 (29.9)	229 (43.1)
	Unknown/missing, n	81	104	143
Dietary supplement use ³ , n (%)	Any	179 (29.7)	212 (37.2)	291 (57.1)
	Containing folic acid	65 (10.8)	105 (18.4)	172 (33.7)
	Unknown supplement use, n	72	105	164
Total folate concentration (nmol/l), median (IQR)		8.5 (6.3-9.8)	15.0 (13.2-17.4)	30.9 (24.5-45.3)
Participants with detectable folic acid concentrations ^{4,5} , n (%)		33 (4.9)	69 (10.2)	199 (29.5)
	Detectable folic acid concentrations (nmol/l), median (IQR)	0.7 (0.6-1.0)	0.8 (0.6-1.2)	1.2 (0.8-2.3)
Participants with detectable pABG concentrations ⁶ , n (%)		653 (96.7)	639 (94.7)	654 (97.0)
	Detectable pABG concentrations (nmol/l), median (IQR)	3.3 (1.5-6.0)	2.3 (0.9-5.5)	1.8 (0.8-4.9)
Participants with detectable apABG concentrations ⁷ , n (%)		581 (86.1)	599 (88.7)	621 (92.1)
	Detectable apABG concentrations (nmol/l), median (IQR)	0.6 (0.4-0.8)	0.7 (0.5-0.9)	0.9 (0.7-1.3)
Total energy intake (kcal/day), median (IQR)		1867 (1562-2279)	1944 (1612-2355)	1807 (1462-2199)
	Unknown/missing, n	94	169	282
Alcohol intake (g/day), median (IQR)		7.6 (1.0-20.5)	9.0 (1.4-20.7)	8.0 (0.3-21.0)
	Unknown/missing, n	79	113	201
Follow-up time ⁸ , median (range)		3.8y (23d-15.4y)	3.6y (14d-15.2y)	3.6y (4d-14.8y)
Deceased ⁹ , n (%)		98 (14.5)	100 (14.8)	90 (13.4)
Recurrence ¹⁰ , n (%)	Yes	84 (12.5)	83 (12.6)	91 (14.2)
	Unknown/missing, n	3	15	28
Location of the recurrence, n (%)	Locoregional	25 (3.7)	18 (2.7)	23 (3.6)
	Distant	75 (11.2)	68 (10.3)	71 (11.2)
	Unknown location	1 (0.2)	3 (0.5)	3 (0.5)
Disease-free survival ¹¹ , n (%)	Participants with at least one event	138 (20.5)	150 (22.8)	140 (21.8)

¹Tumor location is defined as colon (cecum, appendix and ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon) and rectal (rectosigmoid junction and rectum) cancer.

²At least 150 minutes per week of moderate-to-vigorous physical activity.

³Dietary supplement use is defined as the use of single micronutrients and/or the use of multivitamins in the last year for COLON and EnCoRe and in the last month for the ColoCare sites. Dietary supplement use was not collected in the CORSA study.

⁴Pearson correlation coefficient between log2 transformed folate concentrations and log2 transformed folic acid concentrations is 0.45 (n=301).

⁵Detectable folic acid concentrations > 0.52nmol/l.

⁶Detectable pABG concentrations > 0.08nmol/l.

⁷Detectable apABG concentrations > 0.13nmol/l.

⁸Follow-up time calculated using overall survival.

⁹Overall survival events were investigated by using death from any cause in the analysis.

¹⁰Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection

¹¹Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis.

Supplementary Table S2 Baseline characteristics of participants with and without detectable folic acid concentrations and stratified by tertiles of folic acid

	Participants without detectable folic acid concentrations (n=1723)	Participants with detectable folic acid concentrations (n=301)	Folic acid tertiles		
			Tertile 1 (n=100)	Tertile 2 (n=101)	Tertile 3 (n=100)
Men, n (%)	1139 (66.1)	165 (54.8)	57 (57.0)	55 (54.5)	53 (53.0)
Age at diagnosis (years), median (IQR)	66.0 (60.0-72.5)	67.0 (60.1-73.4)	67.3 (61.7-73.3)	66.8 (62.4-73.9)	66.4 (59.8-73.0)
Body mass index (kg/m ²), median (IQR)	26.5 (24.2-29.4)	26.5 (23.9-29.9)	26.3 (23.5-29.4)	25.8 (24.1-29.0)	27.7 (23.8-31.5)
Underweight, <18.5, n (%)	15 (0.9)	2 (0.7)	0 (0.0)	2 (2.0)	0 (0.0)
Normal weight, 18.5-24.9, n (%)	576 (33.8)	108 (36.6)	39 (40.6)	38 (38.0)	31 (31.3)
Overweight, 25-29.9, n (%)	751 (44.1)	112 (38.0)	35 (36.5)	43 (43.0)	34 (34.3)
Obese, ≥30, n (%)	360 (21.2)	73 (24.8)	22 (22.9)	17 (17.0)	34 (34.3)
Unknown/missing, n	21	0	4	1	1
Smoking, n (%)					
Current	215 (13.1)	36 (12.6)	11 (11.6)	15 (15.8)	10 (10.5)
Former	876 (53.4)	152 (53.3)	53 (55.8)	48 (50.5)	51 (53.7)
Never	549 (33.5)	97 (34.0)	31 (32.6)	32 (33.7)	34 (35.8)
Unknown/missing, n	85	16	5	6	5
Stage of disease, n (%)					
I	451 (26.2)	92 (30.6)	36 (36.0)	28 (27.7)	28 (28.0)
II	508 (29.5)	91 (30.2)	24 (24.0)	32 (31.7)	35 (25.0)
III	719 (41.7)	105 (34.9)	36 (36.0)	36 (35.6)	33 (33.0)
Unspecified/unknown	45 (2.6)	13 (4.3)	4 (4.0)	5 (4.9)	4 (4.0)
Tumor location ¹ , n (%)					
Colon	1069 (62.2)	183 (61.0)	62 (62.0)	67 (66.3)	54 (54.6)
Rectal	651 (37.9)	117 (39.0)	38 (38.0)	34 (33.7)	45 (45.4)
Unknown/missing, n	3	1	0	0	1
Neo-adjuvant treatment, n (%)					
Yes	395 (23.0)	63 (20.9)	20 (20.0)	19 (18.8)	24 (24.0)
No	1326 (77.0)	238 (79.1)	80 (80.0)	82 (81.2)	76 (76.0)
Unknown/missing, n	2	0	0	0	0
Surgery, n (%)					

Adjuvant treatment, n (%)	Yes	1672 (97.2)	291 (97.0)	95 (95.0)	100 (99.0)	96 (97.0)
	No	48 (2.8)	9 (3.0)	5 (5.0)	1 (1.0)	3 (3.0)
	Unknown/missing, n	3	1	0	0	1
Cohort, n (%)	Yes	520 (30.5)	76 (25.7)	21 (21.4)	25 (25.8)	30 (30.9)
	No	1186 (69.5)	220 (74.3)	77 (78.6)	76 (75.3)	67 (69.1)
	Unknown/missing, n	17	5	2	0	3
Adherence to physical activity guidelines², n (%)	COLON	940 (54.6)	154 (51.2)	64 (64.0)	57 (56.4)	33 (33.0)
	EnCoRe	250 (14.5)	47 (15.6)	24 (24.0)	13 (12.9)	10 (10.0)
	CORSA	174 (10.1)	35 (11.6)	12 (12.0)	9 (8.9)	14 (14.0)
	ColoCare HD	246 (14.3)	14 (4.7)	0 (0.0)	7 (6.9)	7 (7.0)
	ColoCare HCI	34 (2.0)	12 (4.0)	0 (0.0)	3 (3.0)	9 (9.0)
	ColoCare FHCRC	79 (4.6)	39 (13.0)	0 (0.0)	12 (11.9)	27 (27.0)
	Yes	964 (66.4)	151 (61.6)	53 (63.9)	53 (63.9)	45 (57.0)
	No	487 (33.6)	94 (38.4)	30 (36.1)	30 (36.1)	34 (43.0)
	Unknown/missing, n	272	56	17	18	21
	Dietary supplement use³, n (%)					
Participants with detectable apABG concentrations⁷, n (%)	Any	520 (36.0)	162 (68.4)	53 (63.9)	49 (61.3)	60 (81.1)
	Containing folic acid	225 (25.6)	117 (49.4)	43 (51.8)	34 (42.5)	40 (54.1)
	Unknown supplement use, n	277	64	17	21	26
	Total folate concentration⁴ (nmol/l), median (IQR)	13.9 (9.4-21.1)	27.8 (16.8-46.9)	19.9 (12.6-28.1)	25.8 (16.4-36.5)	49.2 (28.0-79.6)
	Participants with detectable folic acid concentrations⁵, n (%)	0 (0.0)	301 (100.0)	100 (100.0)	101 (100.0)	100 (100.0)
	Detectable folic acid concentrations (nmol/l), median (IQR)	0.0 (0.0-0.0)	1.0 (0.7-1.9)	0.6 (0.6-0.7)	1.0 (0.9-1.2)	2.7 (1.9-5.5)
	Participants with detectable pABG concentrations⁶, n (%)	1645 (95.5)	301 (100.0)	100 (100.0)	101 (100.0)	100 (100.0)
	Detectable pABG concentrations (nmol/l), median (IQR)	2.3 (0.9-4.9)	4.1 (1.6-10.4)	4.6 (1.6-8.2)	4.3 (1.6-11.7)	3.7 (1.5-10.5)
	Participants with detectable apABG concentrations⁷, n (%)	1528 (88.7)	273 (90.7)	85 (85.0)	95 (94.1)	93 (93.0)

Detectable apABG concentrations (nmol/l), median (IQR)	0.7 (0.5-1.0)	0.9 (0.6-1.4)	0.8 (0.6-0.9)	0.9 (0.6-1.2)	1.3 (0.8-2.0)
Total energy intake (kcal/day), median (IQR)	1884 (1564-2279) 457	1860 (1479-2229) 88	1865 (1482-2257) 20	1888 (1548-2302) 28	1846 (1454-2180) 40
Unknown/missing, n					
Alcohol intake (g/day), median (IQR)	8.5 (1.1-21.3) 314	6.4 (0.1-17.7) 79	7.0 (0.1-19.8) 20	8.1 (0.1-17.7) 24	3.3 (0.0-14.2) 35
Unknown/missing, n					
Follow-up time ⁸ , median (range)	3.7y (6d-15.4d) 237 (13.8)	4.0y (4d-14.9y) 51 (16.9)	3.8y (31d-14.3y) 10 (10.0)	4.1y (4d-14.9y) 18 (17.8)	4.1y (48d-14.1y) 23 (23.0)
Deceased ⁹ , n (%)					
Recurrence ¹⁰ , n (%)					
Yes	216 (12.9)	42 (14.2)	8 (8.0)	14 (14.1)	20 (20.2)
Unknown/missing, n	42	4	0	2	4
Location of the recurrence, n (%)					
Locoregional	48 (2.9)	18 (6.1)	3 (3.1)	8 (8.1)	7 (7.1)
Distant	182 (10.9)	32 (10.8)	6 (6.1)	11 (11.1)	15 (15.2)
Unknown location	6 (0.4)	1 (0.3)	0 (0.0)	1 (1.0)	1 (1.0)
Disease-free survival ¹¹ , n (%)					
Participants with at least one event	354 (21.1)	74 (25.0)	15 (15.3)	27 (27.3)	32 (32.3)

¹Tumor location is defined as colon (cecum, appendix and ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon) and rectal (rectosigmoid junction and rectum) cancer.

²At least 150 minutes per week of moderate-to-vigorous physical activity.

³Dietary supplement use is defined as the use of single micronutrients and/or the use of multivitamins in the last year for COLON and EnCoRe and in the last month for the ColoCare sites. Dietary supplement use was not collected in the CORSA study.

⁴Pearson correlation coefficient between log₂ transformed folate concentrations and log₂ transformed folic acid concentrations is 0.45 (n=301).

⁵Detectable folic acid concentrations > 0.52nmol/l.

⁶Detectable pABG concentrations > 0.08nmol/l.

⁷Detectable apABG concentrations > 0.13nmol/l.

⁸Follow-up time calculated using overall survival.

⁹Overall survival events were investigated by using death from any cause in the analysis.

¹⁰Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection.

¹¹Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis.

Supplementary Table S3 Baseline characteristics stratified by tertiles of pABG and apABG

	pABG tertiles			apABG tertiles		
	Tertile 1 (n=647)	Tertile 2 (n=649)	Tertile 3 (n=650)	Tertile 1 (n=600)	Tertile 2 (n=600)	Tertile 3 (n=601)
Men, n (%)	442 (68.3)	420 (74.7)	388 (59.7)	388 (64.7)	387 (64.5)	398 (66.2)
Age at diagnosis (years), median (IQR)	66.0 (59.0-73.0)	66.0 (60.0-72.7)	66.3 (61.2-72.3)	66.0 (60.2-71.9)	65.8 (59.0-72.1)	67.0 (60.0-73.8)
Body mass index (kg/m ²), median (IQR)	27.0 (24.3-29.9)	26.6 (24.2-29.7)	25.8 (23.6-28.7)	26.1 (24.1-29.0)	26.6 (24.2-29.4)	26.8 (24.2-30.0)
Underweight, <18.5, n (%)	6 (1.0)	4 (0.6)	6 (0.9)	5 (0.9)	5 (0.8)	6 (1.0)
Normal weight, 18.5-24.9, n (%)	195 (30.8)	210 (32.7)	256 (39.7)	211 (35.8)	206 (34.7)	180 (30.5)
Overweight, 25-29.9, n (%)	277 (43.7)	276 (43.0)	274 (42.5)	260 (44.1)	256 (43.1)	258 (43.7)
Obese, ≥30, n (%)	156 (24.6)	152 (23.7)	109 (16.9)	113 (19.2)	127 (21.4)	147 (24.9)
Unknown/missing, n	13	7		11	6	10
Smoking, n (%)						
Current	82 (13.6)	82 (13.1)	70 (11.2)	85 (14.5)	71 (12.5)	60 (10.7)
Former	299 (49.7)	358 (57.2)	346 (55.5)	309 (52.8)	312 (55.0)	297 (53.1)
Never	221 (36.7)	186 (29.7)	208 (33.3)	191 (32.7)	184 (32.5)	202 (36.1)
Unknown/missing, n	45	23	26	15	33	42
Stage of disease, n (%)						
I	188 (29.1)	163 (25.1)	171 (26.2)	168 (28.0)	165 (27.5)	154 (25.6)
II	191 (29.5)	192 (29.6)	190 (29.2)	155 (25.8)	176 (29.3)	182 (30.3)
III	249 (38.5)	273 (42.1)	273 (42.0)	261 (43.6)	238 (39.7)	249 (41.4)
Unknown/missing, n	19 (2.9)	21 (3.2)	16 (2.5)	16 (2.7)	21 (3.5)	16 (2.7)
Tumor location ¹ , n (%)						
Colon	389 (60.2)	410 (63.5)	416 (64.0)	391 (65.4)	365 (60.8)	355 (59.3)
Rectal	257 (39.8)	236 (36.5)	234 (36.0)	207 (34.6)	235 (39.2)	244 (40.7)
Unknown/missing, n	1	3	0	2		2
Neo-adjuvant treatment, n (%)						
Yes	130 (20.1)	150 (23.2)	155 (23.9)	131 (21.8)	130 (21.7)	144 (24.0)
No	516 (79.9)	498 (76.9)	495 (76.2)	469 (78.2)	469 (78.3)	457 (76.0)
Unknown/missing, n	1	1	0	0	1	0
Surgery, n (%)						
Yes	621 (33.2)	624 (96.3)	642 (98.9)	571 (95.3)	584 (97.5)	589 (98.2)

Adjuvant treatment, n (%)	Unknown/missing, n		No		24 (3.7)		24 (3.7)		7 (1.1)		28 (4.7)		15 (2.5)		11 (1.8)	
			Unknown/missing, n		2		1		1		1		1		1	
Cohort, n (%)	Yes		212 (33.2)		182 (28.4)		169 (26.2)		177 (29.9)		184 (31.1)		177 (29.7)		177 (29.7)	
	No		426 (66.8)		458 (71.6)		477 (73.8)		416 (70.2)		407 (68.9)		419 (70.3)		419 (70.3)	
Unknown/missing, n			9		9		4		7		9		5		5	
	COLON		171 (26.4)		371 (57.2)		545 (83.9)		391 (65.2)		295 (49.2)		264 (43.9)		264 (43.9)	
EnCoRe			82 (12.7)		151 (23.3)		64 (9.9)		93 (15.5)		103 (17.2)		75 (12.5)		75 (12.5)	
	CORS		139 (21.5)		38 (5.9)		17 (2.6)		95 (15.8)		62 (10.3)		38 (6.3)		38 (6.3)	
ColoCare HD			150 (23.2)		50 (7.7)		13 (2.0)		17 (2.8)		90 (15.0)		122 (20.3)		122 (20.3)	
	ColoCare HCI		27 (4.2)		11 (1.7)		3 (0.5)		2 (0.3)		8 (1.3)		35 (5.8)		35 (5.8)	
Adherence to physical activity guidelines ² , n (%)			78 (12.1)		28 (4.3)		8 (1.2)		2 (0.3)		42 (7.0)		67 (11.2)		67 (11.2)	
	Yes		255 (56.7)		411 (70.6)		434 (71.4)		356 (72.2)		324 (65.2)		293 (57.2)		293 (57.2)	
Unknown/missing, n			195 (43.3)		171 (29.4)		174 (28.6)		137 (27.8)		173 (34.8)		219 (42.8)		219 (42.8)	
	Dietary supplement use ³ , n (%)		197		67		42		107		103		89		89	
Any			148 (33.6)		213 (36.9)		311 (50.9)		173 (35.2)		203 (41.4)		234 (46.3)		234 (46.3)	
	Containing folic acid		39 (8.8)		101 (17.5)		202 (33.1)		95 (19.4)		93 (19.0)		111 (21.9)		111 (21.9)	
Unknown supplement use, n			206		72		39		109		110		95		95	
	Total folate concentration (nmol/l), median (IQR)		18.1 (12.0-28.8)		13.9 (9.5-22.7)		13.7 (8.8-21.3)		11.9 (8.2-17.5)		15.3 (10.5-23.6)		22.2 (13.5-36.6)		22.2 (13.5-36.6)	
Participants with detectable folic acid concentrations ⁴ , n (%)			64 (9.9)		87 (13.4)		150 (23.1)		57 (9.5)		71 (11.8)		145 (24.1)		145 (24.1)	
	Detectable folic acid concentrations (nmol/l), median (IQR)		1.1 (0.7-1.7)		1.2 (0.7-2.2)		0.9 (0.7-1.9)		0.9 (0.6-1.2)		0.8 (0.6-1.1)		1.3 (0.9-2.3)		1.3 (0.9-2.3)	
Participants with detectable pABG concentrations ⁵ , n (%)			647 (100.0)		649 (100.0)		650 (100.0)		584 (97.3)		571 (95.2)		582 (96.8)		582 (96.8)	
	Detectable pABG concentrations (nmol/l), median (IQR)		0.7 (0.5-1.0)		2.5 (1.9-3.2)		7.7 (5.4-11.7)		2.7 (1.0-5.8)		2.4 (1.0-5.0)		2.1 (1.0-5.0)		2.1 (1.0-5.0)	
Participants with detectable apABG concentrations ⁶ , n (%)			588 (90.9)		582 (89.7)		567 (87.2)		600 (100.0)		600 (100.0)		601 (100.0)		601 (100.0)	
	Detectable apABG concentrations (nmol/l), median (IQR)		0.7 (0.5-1.1)		0.7 (0.5-1.0)		0.7 (0.5-1.0)		0.4 (0.4-0.5)		0.7 (0.7-0.8)		1.2 (1.0-1.5)		1.2 (1.0-1.5)	

Total energy intake (kcal/day), median (IQR)	1886 (1528-2301)	1923 (1597-2359)	1836 (1506-2184)	1848 (1550-2245)	1907 (1594-2304)	1881 (1487-2292)
Unknown/missing, n	312	113	62	125	174	194
Alcohol intake (g/day), median (IQR)	7.7 (0.8-21.5)	7.9 (1.1-20.5)	8.5 (1.0-20.8)	7.7 (0.9-19.5)	8.9 (1.4-21.9)	8.5 (0.5-22.0)
Unknown/missing, n	225	84	57	113	121	129
Follow-up time ⁷ , median (range)	3.5y (4d-15.2y)	3.5y (23d-15.2y)	4.3y (25d-15.3y)	4.1y (23d-15.4y)	3.5y (30d-15.2y)	3.3y (4d-14.9y)
Deceased ⁸ , n (%)	104 (16.1)	86 (13.3)	85 (13.1)	79 (13.2)	89 (14.8)	93 (15.5)
Recurrence ⁹ , n (%)						
Yes	86 (13.6)	74 (11.7)	89 (14.0)	73 (12.4)	86 (14.7)	73 (12.5)
Unknown/missing, n				4	17	17
Location of the recurrence, n (%)						
Locoregional	22 (3.5)	12 (1.9)	31 (4.9)	22 (3.8)	20 (3.4)	19 (3.2)
Distant	66 (10.4)	66 (10.4)	74 (11.7)	62 (10.6)	74 (12.7)	58 (9.9)
Unknown location	6 (1.0)	0 (0.0)	1 (0.2)	3 (0.5)	2 (0.3)	3 (0.5)
Disease-free survival ¹⁰ , n (%)						
Participants with at least one event	159 (25.1)	122 (19.2)	129 (20.3)	120 (20.4)	135 (23.1)	132 (22.5)

⁷Tumor location is defined as colon (cecum, appendix and ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon) and rectal (rectosigmoid junction and rectum) cancer.

⁸At least 150 minutes per week of moderate-to-vigorous physical activity.

⁹Dietary supplement use is defined as the use of single micronutrients and/or the use of multivitamins in the last year for CO LON and EnCoRe and in the last month for the ColoCare sites. Dietary supplement use was not collected in the CORSA study.

¹⁰Detectable folic acid concentrations > 0.52 nmol/l.

¹¹Detectable pABG concentrations > 0.08 nmol/l.

¹²Detectable apABG concentrations > 0.13 nmol/l.

¹³Follow-up time calculated using overall survival.

¹⁴Overall survival events were investigated by using death from any cause in the analysis.

¹⁵Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection.

¹⁶Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis.

Supplementary Table S4 Associations between cohort-specific tertiles of circulating concentrations of folate, folic acid, pABG, and apABG and recurrence and survival

Recurrence ¹				Death from any cause (overall survival) ²			Recurrence or death from any cause (disease-free survival) ³		
Median (IQR)	No. of events/at risk	Crude HR (95% CI)	Adj. HR (95% CI) ⁴	No. of deaths/at risk	Crude HR (95% CI)	Adj. HR (95% CI) ⁴	No. of events/at risk	Crude HR (95% CI)	Adj. HR (95% CI) ⁴
Folate (nmol/l)									
T1 ⁵	85/662	1.00 (referent)	1.00 (referent)	103/678	1.00 (referent)	1.00 (referent)	146/662	1.00 (referent)	1.00 (referent)
T2 ⁵	91/665	1.09 (0.81 to 1.47)	1.10 (0.82 to 1.48)	104/676	1.01 (0.77 to 1.32)	0.99 (0.75 to 1.30)	154/665	1.07 (0.85 to 1.34)	1.05 (0.84 to 1.32)
T3 ⁵	82/646	1.00 (0.74 to 1.35)	1.08 (0.80 to 1.47)	81/670	0.73 (0.54 to 0.97)	0.80 (0.60 to 1.07)	128/646	0.86 (0.68 to 1.09)	0.92 (0.73 to 1.17)
P _{trend}		0.98	0.59		0.03	0.14		0.21	0.53
Folic acid (nmol/l)									
T1 ^{6,7}	10/100	1.00 (referent)	1.00 (referent)	12/102	1.00 (referent)	1.00 (referent)	19/100	1.00 (referent)	1.00 (referent)
T2 ^{6,7}	15/101	1.60 (0.72 to 3.57)	1.67 (0.74 to 3.75)	22/103	1.79 (0.89 to 3.63)	2.05 (0.99 to 4.22)	29/101	1.55 (0.87 to 2.76)	1.66 (0.92 to 3.00)
T3 ^{6,7}	17/95	1.87 (0.86 to 4.09)	2.45 (1.09 to 5.49)	17/96	1.41 (0.67 to 2.95)	1.58 (0.74 to 3.38)	26/95	1.47 (0.81 to 2.66)	1.61 (0.88 to 2.97)
P _{trend}		0.12	0.03		0.40	0.26		0.21	0.13
pABG (nmol/l)									
T1 ^{6,7}	82/637	1.00 (referent)	1.00 (referent)	94/650	1.00 (referent)	1.00 (referent)	145/637	1.00 (referent)	1.00 (referent)
T2 ^{6,7}	78/635	0.92 (0.67 to 1.25)	0.94 (0.69 to 1.28)	84/653	0.81 (0.60 to 1.09)	0.87 (0.64 to 1.16)	128/635	0.81 (0.64 to 1.03)	0.84 (0.66 to 1.07)
T3 ^{6,7}	89/632	1.03 (0.77 to 1.40)	1.06 (0.78 to 1.44)	97/643	0.92 (0.70 to 1.23)	1.02 (0.76 to 1.36)	137/632	0.86 (0.68 to 1.08)	0.91 (0.72 to 1.15)
P _{trend}		0.82	0.70		0.60	0.90		0.19	0.42
apABG (nmol/l)									
T1 ^{6,7}	76/587	1.00 (referent)	1.00 (referent)	70/603	1.00 (referent)	1.00 (referent)	116/587	1.00 (referent)	1.00 (referent)
T2 ^{6,7}	84/590	1.14 (0.83 to 1.55)	1.09 (0.80 to 1.49)	92/605	1.35 (0.99 to 1.85)	1.24 (0.91 to 1.70)	140/590	1.24 (0.97 to 1.59)	1.17 (0.91 to 1.50)
T3 ^{6,7}	72/581	1.04 (0.75 to 1.44)	1.04 (0.75 to 1.44)	99/593	1.67 (1.23 to 2.27)	1.42 (1.04 to 1.93)	131/581	1.30 (1.02 to 1.67)	1.19 (0.92 to 1.53)
P _{trend}		0.79	0.82		0.001	0.03		0.04	0.18

¹Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection. A HR > 1.00 should be interpreted as an increased risk of recurrence.
²Overall survival events were investigated by using death from any cause in the analysis. A HR > 1.00 should be interpreted as a reduced overall survival (more deaths).
³Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis. A HR > 1.00 should be interpreted as a reduced disease-free survival (more deaths and/or recurrences).
⁴Adjusted for age, sex, chemotherapy status, and cohort for folate and folic acid. pABG and apABG were additionally adjusted for log2 transformed creatinine concentrations. Stage was tested as potential confounder in all models, but it did not influence the effect estimates.
⁵Tertile cut-off values for folate were 9.6 nmol/l and 16.0 nmol/l for COLON, 13.3 nmol/l and 22.4 nmol/l for EnCoRe, 10.6 nmol/l and 16.5 nmol/l for CORSA, 18.1 nmol/l

and 26.7 nmol/l for ColoCare HD, 51.0 nmol/l and 80.8 nmol/l for ColoCare HCl, 35.2 nmol/l and 61.0 nmol/l for ColoCare FHCRC; analysis performed using non-log₂ transformed concentrations.

⁶Detectable concentrations > 0.52 nmol/l for folic acid, > 0.08 nmol/l for pABG, and > 0.13 nmol/l for apABG.

⁷Analysis only performed for participants with detectable folic acid, pABG, or apABG; analysis performed using non-log₂ transformed concentrations.

Tertile cut-off values for folic acid were 0.7 nmol/l and 1.0 nmol/l for COLON, 0.7 nmol/l and 0.9 nmol/l for EnCoRe, 0.7 nmol/l and 2.4 nmol/l for CORSA, 1.4 nmol/l and 1.7 nmol/l for ColoCare HD, 1.6 nmol/l and 3.6 nmol/l for ColoCare HCl, 1.5 nmol/l and 3.0 nmol/l for ColoCare FHCRC.

Tertile cut-off values for pABG were 2.7 nmol/l and 6.5 nmol/l for COLON, 1.6 nmol/l and 3.2 nmol/l for EnCoRe, 0.5 nmol/l and 1.2 nmol/l for CORSA, 0.7 nmol/l and 1.4 nmol/l for ColoCare HD, 0.6 nmol/l and 1.4 nmol/l for ColoCare HCl, 0.7 nmol/l and 1.3 nmol/l for ColoCare FHCRC.

Tertile cut-off values for apABG were 0.5 nmol/l and 0.8 nmol/l for COLON, 0.6 nmol/l and 0.8 nmol/l for EnCoRe, 0.5 nmol/l and 0.7 nmol/l for CORSA, 0.8 nmol/l and 1.1 nmol/l for ColoCare HD, 1.0 nmol/l and 1.6 nmol/l for ColoCare HCl, 0.8 nmol/l and 1.4 nmol/l for ColoCare FHCRC.

¹⁰LOD: level of detection.

Supplementary Table S5 Associations between circulating concentrations of folate, folic acid, pABG, and apABG and recurrence and survival excluding participants of whom blood was collected during or after any type of treatment

	Recurrence ¹			Death from any cause (overall survival) ²			Recurrence or death from any cause (disease-free survival) ³			
	Median (IQR)	No. of events/at risk	Crude HR (95% CI)	Adj. HR (95% CI) ⁴	No. of deaths/at risk	Crude HR (95% CI)	Adj. HR (95% CI) ⁴	No. of events/at risk	Crude HR (95% CI)	Adj. HR (95% CI) ⁴
Folate (nmol/l)										
Continuous ⁵	14.8 (9.6 to 23.3)	212/1711	1.04 (0.91 to 1.19)	1.03 (0.88 to 1.21)	245/1749	0.98 (0.87 to 1.12)	0.93 (0.80 to 1.08)	359/1711	1.01 (0.91 to 1.12)	0.98 (0.86 to 1.11)
T1 ⁶	8.4 (6.2 to 9.6)	73/570	1.00 (referent)	1.00 (referent)	84/583	1.00 (referent)	1.00 (referent)	119/570	1.00 (referent)	1.00 (referent)
T2 ⁶	14.9 (13.0 to 17.3)	62/571	0.86 (0.61 to 1.20)	0.87 (0.62 to 1.23)	85/583	1.01 (0.74 to 1.36)	0.93 (0.68 to 1.26)	120/571	0.99 (0.77 to 1.28)	0.95 (0.73 to 1.23)
T3 ⁶	29.5 (23.7 to 41.0)	77/570	1.06 (0.77 to 1.46)	1.09 (0.76 to 1.56)	76/583	0.90 (0.66 to 1.23)	0.80 (0.57 to 1.13)	120/570	0.97 (0.75 to 1.25)	0.90 (0.68 to 1.20)
P _{trend}			0.45	0.41		0.45	0.22		0.99	0.69
Folic acid (nmol/l)										
Undetectable	<0.52 (LOD ¹⁰)	117/1452	1.00 (referent)	1.00 (referent)	202/1485	1.00 (referent)	1.00 (referent)	297/1452	1.00 (referent)	1.00 (referent)
Detectable ⁷	0.9 (0.7 to 1.8)	35/259	1.12 (0.78 to 1.61)	1.20 (0.83 to 1.74)	43/264	1.18 (0.85 to 1.63)	1.24 (0.89 to 1.73)	62/259	1.17 (0.89 to 1.53)	1.23 (0.93 to 1.62)
Continuous ⁸	0.9 (0.7 to 1.8)	35/259	1.26 (1.06 to 1.49)	1.33 (1.10 to 1.61)	43/264	1.22 (1.02 to 1.45)	1.07 (0.88 to 1.30)	62/259	1.21 (1.04 to 1.40)	1.16 (0.99 to 1.36)
T1 ⁹	0.6 (0.6 to 0.7)	5/86	1.00 (referent)	1.00 (referent)	8/88	1.00 (referent)	1.00 (referent)	11/86	1.00 (referent)	1.00 (referent)
T2 ⁹	0.9 (0.8 to 1.1)	14/87	3.02 (1.09 to 8.40)	3.76 (1.29 to 10.91)	16/89	1.99 (0.85 to 4.66)	1.61 (0.67 to 3.83)	24/87	2.22 (1.09 to 4.55)	2.41 (1.15 to 5.07)
T3 ⁹	2.6 (1.8 to 5.5)	16/86	3.28 (1.20 to 8.96)	4.48 (1.40 to 14.34)	19/87	2.46 (1.08 to 5.62)	1.48 (0.60 to 3.66)	27/86	2.56 (1.27 to 5.16)	2.54 (1.15 to 5.63)
P _{trend}			0.08	0.07		0.07	0.71		0.04	0.12
pABG (nmol/l)										
Undetectable	<0.08 (LOD ¹⁰)	6/45	1.00 (referent)	1.00 (referent)	9/52	1.00 (referent)	1.00 (referent)	12/45	1.00 (referent)	1.00 (referent)
Detectable ⁷	2.6 (1.1 to 5.5)	206/1666	0.95 (0.42 to 2.13)	1.00 (0.43 to 2.33)	236/1697	0.72 (0.37 to 1.40)	0.95 (0.47 to 1.89)	347/1666	0.78 (0.44 to 1.39)	0.96 (0.53 to 1.74)
Continuous ⁸	2.6 (1.1 to 5.5)	206/1666	1.03 (0.95 to 1.12)	1.04 (0.94 to 1.15)	236/1697	0.93 (0.86 to 1.01)	1.02 (0.93 to 1.13)	347/1666	0.96 (0.90 to 1.02)	1.00 (0.92 to 1.08)
T1 ⁹	0.7 (0.5 to 1.1)	69/556	1.00 (referent)	1.00 (referent)	86/564	1.00 (referent)	1.00 (referent)	134/556	1.00 (referent)	1.00 (referent)
T2 ⁹	2.6 (1.9 to 3.3)	62/555	0.93 (0.66 to 1.32)	0.94 (0.65 to 1.38)	76/568	0.98 (0.72 to 1.34)	1.29 (0.91 to 1.82)	104/555	0.88 (0.68 to 1.14)	0.98 (0.73 to 1.30)
T3 ⁹	7.9 (5.5 to 12.3)	75/555	1.06 (0.76 to 1.47)	1.05 (0.71 to 1.55)	74/565	0.78 (0.57 to 1.07)	1.17 (0.79 to 1.72)	109/555	0.81 (0.63 to 1.05)	0.94 (0.69 to 1.27)
P _{trend}			0.62	0.68		0.09	0.75		0.14	0.66
apABG (nmol/l)										
Undetectable	<0.13 (LOD ¹⁰)	22/191	1.00 (referent)	1.00 (referent)	23/198	1.00 (referent)	1.00 (referent)	34/190	1.00 (referent)	1.00 (referent)
Detectable ⁷	0.7 (0.5 to 1.0)	190/1520	1.17 (0.75 to 1.82)	1.22 (0.78 to 1.92)	222/1551	1.40 (0.91 to 2.15)	1.24 (0.80 to 1.92)	325/1520	1.32 (0.93 to 1.88)	1.25 (0.87 to 1.80)

Continuous ⁸	0.7 (0.5 to 1.0)	190/1520	0.95 (0.79 to 1.15)	0.92 (0.75 to 1.14)	222/1551	1.21 (1.03 to 1.43)	1.08 (0.89 to 1.30)	325/1520	1.10 (0.96 to 1.27)	1.00 (0.85 to 1.17)
T1 ⁹	0.4 (0.4 to 0.5)	62/506	1.00 (referent)	1.00 (referent)	68/518	1.00 (referent)	1.00 (referent)	101/506	1.00 (referent)	1.00 (referent)
T2 ⁹	0.7 (0.6 to 0.8)	73/507	1.23 (0.88 to 1.73)	1.21 (0.85 to 1.71)	76/516	1.33 (0.96 to 1.85)	1.29 (0.92 to 1.80)	116/507	1.26 (0.96 to 1.64)	1.23 (0.93 to 1.61)
T3 ⁹	1.2 (1.0 to 1.5)	55/507	0.97 (0.67 to 1.40)	0.95 (0.64 to 1.41)	78/517	1.55 (1.12 to 2.15)	1.28 (0.89 to 1.83)	108/507	1.31 (1.00 to 1.72)	1.15 (0.85 to 1.54)
P _{trend}			0.75	0.66		0.01	0.23		0.06	0.47

¹Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection. A HR > 1.00 should be interpreted as an increased risk of recurrence.

²Overall survival events were investigated by using death from any cause in the analysis. A HR > 1.00 should be interpreted as a reduced overall survival (more deaths).

³Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis. A HR > 1.00 should be interpreted as a reduced disease-free survival (more deaths and/or recurrences).

⁴Adjusted for age, sex, chemotherapy status, and cohort for folate and folic acid. pABG and apABG were additionally adjusted for log₂ transformed creatinine concentrations. Stage was tested as potential confounder in all models, but it did not influence the effect estimates.

⁵Analysis performed using log₂ transformed concentrations. Thus, HRs represent a doubling in folate concentrations.

⁶Tertile cut-off values for folate were 11.2 nmol/l and 19.8 nmol/l; analysis performed using non-log₂ transformed concentrations

⁷Detectable concentrations > 0.52 nmol/l for folic acid, > 0.08 nmol/l for pABG, and > 0.13 nmol/l for apABG; analysis performed using log₂ transformed concentrations.

⁸Analysis only performed in the participants with a detectable value; analysis performed using log₂ transformed concentrations. HRs thus represent a doubling in concentrations.

⁹Analysis only performed for participants with detectable folic acid, pABG, or apABG. Tertile cut-off values were 0.71 nmol/l and 1.32 nmol/l for folic acid, 1.45 nmol/l and 4.26 nmol/l for pABG, and 0.57 nmol/l and 0.86 nmol/l for apABG; analysis performed using non-log₂ transformed concentrations.

¹⁰LOD: level of detection.

Supplementary Figure S1 Dose-response relationship of folate concentration (nmol/l) by cohort for recurrence, overall and disease to free survival. Hazard Ratios (HR) and corresponding 95% confidence intervals are shown for cohort to specific tertiles with the lowest tertile as the reference. Arrows represent an upper limit above a HR of 3.00 or a lower limit close to a HR of 0.00.

Supplementary Figure S2 Forest plots of subgroup analyses reporting hazard ratios and corresponding 95% CI for a doubling in pABG concentrations and recurrence, disease-free and overall survival. Weights of the effect estimates are from random effects meta-analysis; square dots represent the hazard ratio of each subgroup while the diamonds represent the hazard ratio of all subgroups combined. Heterogeneity among subgroups was evaluated using the I^2 index.

Supplementary Figure S3 Forest plots of subgroup analyses reporting hazard ratios and corresponding 95% CI for a doubling in apABG

concentrations and recurrence, disease-free and overall survival. Weights of the effect estimates are from random effects meta-analysis; square dots represent the hazard ratio of each subgroup while the diamonds represent the hazard ratio of all subgroups combined. Heterogeneity among subgroups was evaluated using the I^2 index.

Chapter 6

General discussion

In this thesis, different research aims are addressed **to improve our understanding of how metabolites are involved in the colorectal cancer continuum from a clinical and nutritional perspective**. The aims of the current thesis are:

1. To investigate which circulating metabolites are associated with colorectal cancer occurrence and colorectal cancer stage.
2. To explore the association between dietary exposures and circulating metabolites in colorectal cancer patients.
3. To assess the association between circulating folate and folic acid concentrations and recurrence and survival in colorectal cancer patients.

Following the summary of the main findings, methodological considerations, including data harmonization of cohort studies and metabolomics approaches, and biological considerations will be addressed. The generalizability of the findings will be reviewed, and overall conclusions will be drawn. Future research perspectives and consortia of the future will be discussed. The general discussion will end with concluding remarks considering implications for (future) patients and clinical practice.

Main findings

Table 1 provides an overview of the main findings of this thesis, which will be discussed below in more detail from a clinical and a nutritional perspective.

Colorectal cancer: A clinical perspective

Aim 1: The association between circulating metabolites and colorectal cancer occurrence and colorectal cancer stage.

In **Chapters 2 and 3**, the associations between plasma metabolites and colorectal cancer occurrence and stage are studied. In **Chapter 2**, 28 plasma metabolites are reported to be associated with colorectal cancer, when comparing colorectal cancer patients with colorectal cancer-free individuals. An untargeted metabolomics approach is used in combination with a discovery-replication strategy. Consistent findings are observed in the two independent study populations, i.e. the discovery and replication population. Fifteen out of the 28 observed metabolites are known compounds; taurine, hypoxanthine, lysophosphatidylethanolamines (LysoPEs) (20:4) and (22:6) are shown to be higher among

colorectal cancer patients compared to the colorectal cancer-free individuals. The opposite is observed for valine, leucine, bilirubin, 1-methylnicotinamide, and seven phosphatidylcholines (LysoPCs), namely LysoPC(15:0), LysoPC(16:0), LysoPC(16:0) isomer, LysoPC(P-16:0), LysoPC(16:1), LysoPC(17:0), and LysoPC(18:0), which show lower levels among colorectal cancer patients compared to colorectal cancer-free individuals.

Taurine, hypoxanthine, valine, leucine, and LysoPCs have been linked to colorectal cancer occurrence in other studies [1-10] and the directions of the associations are, generally, comparable to the observations in the current thesis. In line with our studies, higher tissue and serum levels of taurine [4, 6] and lower levels of valine, leucine, and LysoPCs are reported among colorectal cancer patients compared to colorectal cancer-free individuals [1-3, 5, 7-10]. In this thesis, higher plasma levels of hypoxanthine are reported for colorectal cancer patients compared to colorectal cancer-free individuals, which was also observed in tumor tissue compared to normal colon tissue of colorectal cancer patients [2]. However, another study showed lower serum levels of hypoxanthine among colorectal cancer patients compared to individuals without a history of cancer [11]. In summary, there is consistency among our and previous studies in reported associations between amino acids and lipids and colorectal cancer occurrence, suggesting amino acid and lipid metabolism is associated with colorectal cancer occurrence.

In **Chapter 3**, we report that plasma metabolites at cancer diagnosis are associated with colorectal cancer stage. Plasma sphingomyelin (SM) C26:0 showed lower concentrations among stage III compared to stage I colorectal cancer patients. When comparing stage IV with stage I colorectal cancer patients, SM C18:0 and phosphatidylcholine diacyl (PC aa) C32:0 concentrations are higher, whereas citrulline, histidine, PC aa C34:4, PC acyl-alkyl (ae) C40:1 and LysoPC a C16:0 and C17:0 are lower among stage IV patients. No differences in plasma metabolite concentrations are observed between stage II and stage I colorectal cancer patients. Comparison with previous studies is challenging, since earlier studies usually combined stages into early (stage I-II or stage I-III) and late-stage (stage III-IV or stage IV) colorectal cancer [3, 12, 13], while individual colorectal cancer stages are investigated in this thesis. Previous studies were usually relatively small in sample size [3, 13], and used untargeted or targeted approaches covering different metabolites compared to this thesis [3, 12, 13]. Metabolite classes such as amino acids and lipids have been consistently reported in earlier studies [3, 13, 14] and this thesis. Therefore, metabolites present in amino acid and lipid metabolism, in line with the findings

related to colorectal cancer occurrence, seem of interest when investigating the association between metabolites and colorectal cancer stage.

Colorectal cancer: A nutritional perspective

Aim 2: The association between dietary exposures and metabolites in colorectal cancer patients.

In colorectal cancer patients, better adherence to healthier diets is, in general, associated with lower concentrations of phosphatidylcholines (**Chapter 4**). More specifically, patients with better adherence to the WCRF dietary score, DHD guidelines and higher consumptions of the Prudent patterns show mostly lower concentrations of plasma phosphatidylcholines. In line with these findings, patients with higher intakes of the Western and Carnivore pattern show mostly higher concentrations of plasma phosphatidylcholines. Previous research is limited; two earlier studies among cancer-free individuals reported lower circulating phosphatidylcholine concentrations with higher consumption of a Mediterranean diet [15] and vegan diet [16] compared to a diet with high intakes of animal products. Based on **Chapter 4** and other studies [15, 16], a healthier diet seems to be associated with lower circulating phosphatidylcholine concentrations. Taken together with observations from **Chapters 2 and 3**, where lipid metabolism also appeared to be of interest, investigating the role of lipid metabolism in the colorectal cancer continuum may be considered important from a clinical and nutritional perspective. The used methods in **Chapter 3 and 4** are mainly focused on the measurement of lipid species, which could be one explanation why specifically lipid metabolism is consistently identified as a key process in this thesis, an issue that will be discussed later, see *Metabolomics approaches*.

Table 4 Overview of the main findings described in this thesis

	Chapter 2	Chapter 3	Chapter 4	Chapter 5
<i>Perspective Aim</i>	Clinical	Clinical	Nutritional	Nutritional
<i>Exposures</i>	To compare metabolites between colorectal cancer patients and colorectal cancer-free individuals. Circulating plasma features ¹	To compare metabolites between individual colorectal cancer stages. Circulating plasma metabolites	To investigate associations between diet and metabolites in colorectal cancer patients. Diet quality indices (WCRF, DHDI5-index), dietary patterns (Western, Carnivore, Prudent) Circulating plasma metabolites	To investigate the association between folate species and colorectal cancer outcomes. Circulating folate, folic acid, and folate catabolites pABG and apABG
<i>Outcomes</i>	Colorectal cancer occurrence	Colorectal cancer stage		Colorectal cancer recurrence and survival
<i>Metabolite measurement approach</i>	Untargeted (UHPLC-QTOF-MS)	Targeted (UHPLC-MS/MS, Biocrates AbsoluteIDQ™ p180 Kit)	Targeted (UHPLC-MS/MS, Biocrates AbsoluteIDQ™ p180 Kit)	Targeted (LC-MS/MS optimized for folate species quantification)
<i>Main findings²</i>	<u>Colorectal cancer patients compared to colorectal cancer-free individuals</u> ↓ : Leucine, MNA, valine, bilirubin, LysoPC(16:1), LysoPC(P-16:0), LysoPC(15:0), LysoPC(16:0), LysoPC(16:0) isomer, LysoPC(17:0), LysoPC(18:0), and ten unidentified features concentrations. ↑ : Taurine, hypoxanthine, LysoPE(20:4), LysoPE(22:6), and three unidentified features concentrations.	<u>Stage II compared to stage I</u> No associations. <u>Stage III compared to stage I</u> ↓ : SM C26:0 concentrations. <u>Stage IV compared to stage I</u> ↓ : Citrulline, LysoPC a C16:0, LysoPC a C17:0, PC aa C24:4, PC ae C40:1 and histidine concentrations. ↑ : PC aa C32:0 and SM C18:0 concentrations.	<u>A high compared to low WCRF dietary score</u> ↓ : PC ae C36:2, PC ae C36:4, PC aa C36:3, PC aa C38:3, PC ae C34:3, PC ae C38:4, PC aa C40:4, PC ae C38:3, PC ae C38:5 and PC ae C40:4 concentrations. <u>High compared to low consumption of the Carnivore pattern</u> ↑ : PC aa C38:0 and PC ae C38:6 concentrations. <u>DHDI5-index, Western and Prudent pattern</u> No associations between the DHDI5-index, Western and Prudent pattern and metabolites.	<u>Colorectal cancer recurrence</u> Higher compared to lower circulating folic acid concentrations are associated with more recurrences. No associations between circulating folate, pABG, and apABG concentrations and recurrence. <u>Colorectal cancer survival</u> No associations between circulating folic acid, folate, pABG, and apABG concentrations and survival.

Abbreviations: UHPLC: ultra-high performance liquid chromatography, QTOF: quadrupole time-of-flight, MS: mass spectrometry, MNA: 1-methylnicotinamide, LysoPCs: lysophosphatidylcholines, LysoPEs: lysophosphatidylethanolamines, SM: sphingomyelin, PC aa/ae: phosphatidyl diacyl/acyl-alkyl, WCRF: World Cancer Research Fund, DHDI 5-index: Dutch Healthy Diet index 2015, pABG: p-aminobenzoylethylglutamate, apABG: p-acetamidobenzoylethylglutamate.

Footnotes: ¹Feature refers to unidentified molecules as measured by untargeted techniques. After statistical analysis, features are annotated as specific metabolites; ²Reported associations are statistically significant, ↑: increased circulating concentrations, ↓: decreased circulating concentrations.

Abbreviations: UHPLC: ultra-high performance liquid chromatography, QTOF: quadrupole time-of-flight, MS: mass spectrometry, MNA: 1-methylnicotinamide, LysoPCs: lysophosphatidylcholines, LysoPEs: lysophosphatidylethanolamines, SM: sphingomyelin, PC aa/ae: phosphatidyl diacyl/acyl-alkyl, WCRF: World Cancer Research Fund, DHDI5-index: Dutch Healthy Diet index 2015, pABG: p-aminobenzoylglutamate, apABG: p-acetamidobenzoylglutamate.

Footnotes: ¹Feature refers to unidentified molecules as measured by untargeted techniques. After statistical analysis, features are annotated as specific metabolites; ²Reported associations are statistically significant, ↑: increased circulating concentrations, ↓: decreased circulating concentrations.

Aim 3: The association between folate and folic acid concentrations and recurrence and survival in colorectal cancer patients

The associations between circulating concentrations of folate, folic acid, and folate catabolites pABG and apABG at cancer diagnosis and colorectal cancer recurrence and survival are investigated in **Chapter 5**. Folate, pABG and apABG concentrations are not associated with colorectal cancer recurrence and survival in this thesis. However, higher compared to lower concentrations of folic acid, i.e. synthetic folate, are associated with more recurrences. Folic acid concentrations are not statistically significantly associated with colorectal cancer survival. Earlier studies investigating different folate species in relation to colorectal cancer prognosis are scarce. The absence of an association between folate concentrations and colorectal cancer recurrence and survival is consistent with one earlier study among stage I-III colorectal cancer patients [17]. No previous studies investigated the association between circulating folic acid and folate catabolites concentrations and colorectal cancer recurrence and survival. Fortification of flour and cereal grains is implemented in up to eighty countries worldwide [18] and folic acid is usually present in dietary multivitamins. Because of the speculated harmful effect of excessive intakes of folic acid on colorectal cancer risk [19, 20], establishing the role of synthetic folate in colorectal cancer progression is important for future studies.

Methodological considerations

Data harmonization across cohort studies

Data from several prospective cohort studies from Europe and the US are combined in this thesis, as part of the MetaboCCC and FOCUS consortia. The variability in exposures and outcomes may be higher compared to the variability in a single cohort study. As a result, generalizability of the consortia for Western colorectal cancer patients is higher compared to the potential generalizability of a single cohort study.

To be able to perform a multi-cohort analysis, demographical, clinical, nutritional, and lifestyle information of study participants need to be harmonized across cohorts. The harmonization of data from cohorts is highly time-consuming and challenging; however, it is a crucial step in international consortia, which is often underestimated [21]. At the start of the collaboration, an inventory of the study designs, data collection methods, and all available

data of each cohort must be prepared. After establishing potential overlap in the available data on participants, each item needs to be examined in detail to investigate whether harmonization is possible. For this thesis, it was possible to harmonize most of the essential information, such as cancer stage, smoking status, date of cancer diagnosis and date of blood collection.

As within most consortia in which existing cohort studies are combined, study designs and data collection methods of the cohort studies were not identical. To illustrate, blood samples collected at cancer diagnosis of one cohort were always collected prior to any treatment, while for the other cohorts it was possible that blood samples at diagnosis were taken after the start of neo-adjuvant treatment. As a result, patients with blood samples collected after any treatment were excluded from analysis in **Chapter 2**, resulting in a smaller sample size. For **Chapters 3-5**, patients with blood samples collected after any treatment were excluded in a sensitivity analysis, in which it was shown those blood samples did not substantially influence the findings in this thesis. However, since some minor differences in effect sizes are observed in **Chapters 3 and 5**, it would be preferable to align study designs and data collection methods and collect all blood samples prior to any treatment in future studies.

Although most cohorts collected information on whether patients received neo- and/or adjuvant treatment, details on the exact timing of drawing the blood sample with respect to the receipt of treatment was not (yet) collected in all cohorts. Moreover, fasting status was not known for all participants in all cohorts, which makes it impossible to disentangle the potential influence of fasting status in the presented studies. Fasting status in **Chapter 2** did not differ between colorectal cancer patients and colorectal cancer-free individuals and should not have influenced the findings. In **Chapter 3**, most of the cohort-specific analyses yielded similar results compared to the results of the total study population, thus the potential influence of fasting status on the association between metabolite concentrations and colorectal cancer stage is assumed to be minimal in this study.

In **Chapter 4**, in which the associations between dietary exposures and plasma metabolites are investigated, only one cohort was used. In this cohort, fasting status was unavailable and, therefore, it was not possible to take this into account. It is possible that some of the presented results in **Chapter 4** are related to recent dietary intake for some participants [22]. Fasting status did not influence the associations between dietary patterns

and amino acids and phosphatidylcholines concentrations in another recent study [23] and fasting status is suggested to mainly influence metabolite classes such as acylcarnitines [22]. The observed associated metabolites with colorectal cancer, colorectal cancer stage and dietary exposures in this thesis were predominantly phosphatidylcholines and amino acids (**Chapters 2-4**), therefore, we assume the influence of fasting status to be low in **Chapters 2-4**. Fasting status may have influenced the quantification of folate species by mass spectrometry (**Chapter 5**). Concentrations of folate species may have been influenced by a recent intake of a high-dose folic acid-containing dietary supplement or folic acid-fortified food. Therefore, our results regarding the increased risk of colorectal cancer recurrences with increasing folic acid concentrations (**Chapter 5**) may have been affected by fasting status for the participants that were nonfasted.

The quantity of alcohol intake or details on the use of dietary supplements, such as the composition and exact dose of components in the supplements, is not routinely collected for all the included cohorts. Consequently, it was also not possible to address the potential influences of, for example alcohol intake in the analyses of **Chapters 3 and 5** or the role of dietary intake of supplemental B vitamins in **Chapter 5**. In **Chapter 5**, it was not possible to investigate whether the observed positive association between circulating folic acid concentrations and colorectal cancer recurrence is attributable to the potential intake of fortified food or the use of folic acid-containing dietary supplements or a combination of the latter two. Intake of dietary supplements containing folic acid was reported by 20% of the study population, but not all of these participants had detectable folic acid concentrations in their blood sample. Fifteen percent of the study population in **Chapter 5** has a detectable folic acid concentration in their blood sample, but not all reported using dietary supplements at time of blood collection. It is observed that cohorts are equally represented in the fifteen percent with a detectable folic acid concentration. Although the US have mandatory folic acid fortification, the proportion of participants with detectable unmetabolized folic acid concentrations was not higher in the US cohorts compared to the European cohorts. Therefore, acknowledging that there might be some reporting bias regarding dietary supplement use and the fact that voluntary folic acid fortification is present among widely used products, such as breakfast cereals, in countries without mandatory folic acid fortification [24-26], it may be assumed that the observed positive association between circulating folic acid concentrations and colorectal cancer recurrence is likely attributable to

the combination of high intakes of fortified food and the use of high-dose folic acid-containing dietary supplements.

Summarizing, harmonizing data across multiple cohorts has advantages and disadvantages. Major strengths of this thesis are the ability to compare colorectal cancer patients with colorectal cancer-free individuals (**Chapter 2**), the availability of data from colorectal cancer patients from several countries (**Chapters 2, 3 and 5**), and the availability of a wide variability in exposures and outcomes (**Chapters 3 and 5**). In **Chapter 4**, it was, however, decided to focus on one cohort from one country to be able to investigate dietary exposures in detail, as dietary intake collection methods were not identical or not (yet) harmonized across cohorts. For the multi-cohort analyses, the cohort with the least detailed information on their study participants will, often, unintentionally, lead the harmonization. To illustrate, the use of dietary supplements is harmonized to a yes/no variable across the consortia, while some cohorts provided the exact quantity of components in the dietary supplements (**Chapter 5**). In the latter example, a larger sample size was chosen over precision since the exact quantity of supplemental vitamins was not the focus of the analysis. Another solution would have been to choose precision over quantity, which would have significantly reduced the sample size of the analysis in **Chapter 5**. Both possibilities lead to potential limited use of available resources, but the research question of interest will drive the necessity of quantity or quality.

Metabolomics approaches

Several metabolomics approaches are applied in this thesis, namely untargeted metabolomics (**Chapter 2**), targeted metabolomics using the Biocrates *AbsoluteIDQ*^{TM} p180 Kit (**Chapters 3 and 4**) and targeted metabolomics optimized for folate species quantification (**Chapter 5**). A major strength of the studies presented in this thesis is that all blood samples investigated in the MetaboCCC (**Chapters 2-4**) and FOCUS consortia (**Chapter 5**) are measured at the International Agency for Research on Cancer (IARC) and BEVITAL, respectively.

Untargeted metabolomics is based on high resolution mass spectrometry (HRMS). Sample analyses by HRMS generate thousands of signals, known as mass spectrometry (MS) features characteristic of a large diversity of metabolites. The workflow for untargeted metabolomics data analysis includes several steps. Firstly and prior to data analysis, untargeted metabolomics data need pre-processing to align the features over samples and

analytical batches and to reduce potential instrumental noise [27, 28]. There is no standardization to date to decide which methods are most appropriate to reduce potential artifacts and to end up with a reasonable amount of data for further statistical analysis [29]. Secondly, there are a variety of potential statistical approaches to analyze untargeted metabolomics datasets [27]. Thirdly, the annotation of features, i.e. identification of ions, remains one of the main bottlenecks in untargeted metabolomics. Metabolite identification is a time-consuming multistep process which requires authentic chemical standards, that are often are expensive and not always available [30]. Data pre-processing, statistical data analyses, metabolite identification and deciding on the appropriate bioinformatic tools to use are all challenging and non-standardized steps which require diverse expertise are required to decide which workflow is best suited to conduct data analysis [31, 32].

Because of the large datasets generated by untargeted metabolomics, it is extremely valuable to conduct a discovery-replication analysis to investigate the reproducibility of findings. In **Chapter 2**, a workflow is set up to use the data most effectively and to perform a discovery-replication analysis. Comparing results of different studies using untargeted metabolomics techniques is often difficult due to possible bias linked to the mode of sample collection, sample storage and processing, the use of different mass spectrometry instruments, and different workflows for data analyses. In addition, untargeted metabolomics provides relative signal intensities between samples in a particular study (semi-quantitative data) [33]. It is indeed impossible to run calibration curves when so many metabolites are measured. In these conditions, specific quality procedures are needed and have been developed to monitor the stability of the instrument during data acquisition along a given batch made of several hundred samples. When the number of samples to be analyzed exceeds the maximum size of a batch, data from different batches are aligned through correction of the batch effect.

In this thesis, analytical plates refer to 96-well plates containing biological study samples, quality controls and blanks, while a batch is defined as a set of analytical plates that have been measured on the same mass spectrometry instrument in one uninterrupted sequence [34]. Both batches and analytical plates can influence metabolite concentrations [33] and should be considered in data analysis accordingly. A major strength of the presented studies in this thesis (**Chapters 2-4**) is that all study samples are analyzed using the same instrument. In **Chapter 5**, study samples were measured over two time periods. Within the MetaboCCC consortium, an ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) instrument was used at IARC for the

untargeted metabolomics analyses (**Chapter 2**). Study samples all cohorts were analyzed in four separate cohort-specific batches because of the large total sample size. Randomizing all participants from all cohorts was considered, however, this would have undermined the possibility to do multi-cohort analyses as randomized batches would have made it difficult to disentangle whether associations would be due to the outcome of interest or potential cohort effects. Analyzing cohort-specific batches meant that it was not possible to include the total MetaboCCC study population in **Chapter 2** since cohorts are measured in different batches and not all cohorts included both colorectal cancer-free individuals and colorectal cancer patients. Including the total MetaboCCC study population for the analysis in **Chapter 2** would have made it impossible to determine whether observed metabolites would be due to the outcome of interest, i.e. the association between metabolites and cancer occurrence, or whether findings were due to batch effects or cohort-specific associations. In **Chapter 2**, the analysis was, therefore, forced to only include cohorts including colorectal cancer patients as well as colorectal cancer-free individuals.

For **Chapters 3** and **4**, the Biocrates *AbsoluteIDQ*^{TM} kit is used to estimate up to 186 metabolites on a liquid chromatography coupled to a tandem mass spectrometer (UHPLC-MS/MS) at IARC. The Biocrates *AbsoluteIDQ*^{TM} kit consists of a pre-selected number of metabolites, has a high inter-laboratory reproducibility [35] and is frequently used in previous studies [16, 36, 37]. Out of these 186 metabolites, some of these metabolites were either not detected in a sufficient fraction of the samples (>20% missing values) or showed a too high technical variability (CV >20%). These compounds were removed from further analyses, leaving 130 (**Chapter 3**) and 134 (**Chapter 4**) metabolites, respectively, for further analyses. These metabolites were either quantified (amino acids and biogenic amines; calibration curve run with authentic chemical standard for each batch and metabolite) or semi-quantified (acylcarnitines, lipids, sugar; no calibration, relative concentrations).

Within the FOCUS consortium, LC-MS/MS was performed at BEVITAL, where the instrument is optimized to quantify folate species to absolute concentrations (**Chapter 5**). In addition to running all batches and analytical plates after each other, several quality control and blank samples are included to perform quality control checks for potential technical influences within the MetaboCCC and FOCUS consortia prior to analyses. Previous metabolomics studies give relatively little information on whether quality control checks were performed or how different analytical batches and plates were considered in the data

analyses. This thesis investigated the confounding effect of analytical plate in all presented studies, which adds to the validity of the presented findings in **Chapters 2-5**.

In this thesis, lipid (**Chapters 2-4**) and amino acid (**Chapters 2 and 3**) metabolism consistently showed to be of interest, and untargeted analysis (**Chapter 2**) did not reveal many metabolites beyond lipid and amino acid metabolism. However, we cannot exclude that other metabolites might be involved in the colorectal cancer continuum, acknowledging that several features in **Chapter 2** remain to be annotated and untargeted analysis only measures a specific range of molecule masses. Other metabolite classes might be of interest when investigating associations between metabolites and the colorectal cancer continuum from a clinical and nutritional perspective in the future. Potential other metabolite classes are for example metabolites involved in glycolytic pathways [38], endocannabinoid metabolism [39], and tricarboxylic acid cycle-related metabolites, especially since citrulline and histidine concentrations are observed to be lower among stage IV compared to stage I colorectal cancer patients (**Chapter 3**).

In contrast to our study (**Chapter 5**), in which we use targeted metabolomics optimized for folate quantification, previous studies quantified folate concentrations with the microbiological assay using *Lactobacillus casei* [17, 40]. The microbiological assay is, to date, the golden standard to measure folate, but the assay cannot detect individual folate species and is not able to detect low concentrations of folate [41, 42]. Using a targeted LC-MS/MS metabolomics approach, as was done in the FOCUS consortium, is potentially a more potent method to quantify individual folate species concentrations [43].

One limitation of our work was that metabolomics analyses are only performed on a single sample collected at the time of cancer diagnosis, while it might be important to evaluate the reliability of metabolomics measurements over time [44]. Since metabolomics analyses reflect the current metabolic state of the body at the time of sampling, results may be sensitive to physiological and environmental influences, such as fasting status, alcohol intake, medication use, and the receipt of neo- and/or adjuvant treatment, which may vary time of blood collection [45]. Presented results (**Chapters 2-5**) might be influenced by a recent occasional event, which may cause some interindividual differences because of physiological and environmental characteristics of the participants at the time of blood draw. Some of these interindividual differences might be randomly distributed among the study population and are, therefore, not expected to have a major influence on the presented

findings in this thesis. Other influences, such as neo-adjuvant treatment, might significantly influence results, as discussed in the section ‘Data harmonization across cohort studies’, and should be considered as potential confounders and effect measure modifiers, as has been done in this thesis.

Biological considerations

In the current thesis, associations between metabolites and the colorectal cancer continuum are investigated from a nutritional and clinical perspective. Acknowledging that it is not possible to disentangle cause and effect in the presented studies, some biological considerations can be discussed. Lipid species and amino acids are consistently observed to be of relevance in **Chapters 2-4**; lipid and amino acid concentrations were associated with colorectal cancer occurrence (**Chapter 2**), with stage III and IV colorectal cancer (**Chapter 3**), and dietary exposures were associated with phosphatidylcholine concentrations in colorectal cancer patients (**Chapter 4**).

Since blood samples in this thesis are collected, mostly shortly, after cancer diagnosis, the observed findings might reflect an altered lipid metabolism because of the tumor. Lower plasma phosphatidylcholine concentrations are observed among colorectal cancer patients compared to colorectal cancer-free individuals (**Chapter 2**) and among stage III and IV compared to stage I colorectal cancer patients (**Chapter 3**), both supporting the hypothesis of accelerated lipid breakdown as a result of accelerated cell proliferation of cancerous cells [46-48]. Previous studies showed that increased lipid breakdown leads to increased formation of lipoperoxyl radicals, which subsequently degrades to products contributing to oxidative stress [49, 50]. In this thesis, the finding of increased concentrations of hypoxanthine in colorectal cancer patients compared to colorectal cancer-free individuals (**Chapter 2**) may also support the speculative hypothesis about increased lipid breakdown because of the tumor. Hypoxanthine has been reported to contribute to oxidative stress [28] and has been linked to colorectal cancer etiology in the past [51, 52]. Hypoxanthine is not present in the Biocrates *AbsoluteIDQ*^{TM} kit used in **Chapter 3**, therefore, we can only speculate that hypoxanthine levels might be lower among more advanced compared to early stage colorectal cancer patients.

Adherence to a healthier diet seems associated with lower phosphatidylcholine concentrations in colorectal cancer patients (**Chapter 4**), which is in line with what has been

observed earlier in healthy populations [15, 16, 23]. Based on **Chapter 3**, it might be hypothesized that colorectal cancer stage could be confounder and/or effect measure modifier in the association between dietary exposures and phosphatidylcholine concentrations. Adjusting for or stratification by colorectal cancer stage did not show differences in associations between dietary exposures and metabolite concentrations, which may be due to the relatively small sizes in the subgroups (**Chapter 4**).

In addition to altered lipogenesis, reported associations in this thesis hint towards an altered protein turnover. Lower valine and leucine concentrations are observed in colorectal cancer patients compared to colorectal cancer-free individuals (**Chapter 2**), as well as lower concentrations of the amino acids citrulline and histidine in stage IV compared to stage I patients (**Chapter 3**). Lower plasma amino acid concentrations among colorectal cancer patients compared to healthy individuals and among late compared to early stage colorectal cancer patients have been reported in the past [53], but the underlying mechanisms remain unclear.

Malignant cells are suggested to have an increased protein turnover as they need more amino acids to synthesize proteins and nucleic acids [54] and colorectal cancer tumor tissue showed higher amino acid concentrations compared to healthy tissue [2, 55]. The increased energy needs of cancer cells may be a potential explanation for the observed lower amino acid concentrations over the colorectal cancer continuum in this thesis.

Lower amino acid concentrations, specifically citrulline and histidine concentrations in **Chapter 3**, among stage IV compared to stage I colorectal cancer patients may also be a result of increased hepatic uptake as a result of liver metastases [56]. Prior studies suggest that metabolite concentration differences among colorectal cancer stages are mostly expected in patients with metastatic colorectal cancer [12, 13], which seems to be in line with what is observed in **Chapter 3**. This may be reasonable as the tumor size, depth of invasion and potential metastasis may change the tumor metabolism, potentially increasing energy use [57]. Lower amino acid and lipid concentrations because of metastasis may, therefore, be expected, which was also observed in **Chapter 3**.

1-methylnicotinamide (MNA), which is reported to be lower among colorectal cancer patients compared to colorectal cancer-free individuals (**Chapter 2**), is produced in the liver and has been suggested to have anti-inflammatory properties in mice [58]. MNA has also been linked to inflammation in carcinogenesis [59, 60] by the proposed stimulating influence

on the cyclooxygenase (COX)-2/PGI₂ pathway [61]. COX enzymes are essential in the formation of prostaglandins, which play critical regulatory roles in numerous biological processes such as immune function [62]. In colorectal carcinogenesis, MNA is thought to have anti-inflammatory properties by reducing the production of reactive oxygen species in human colorectal cancer cells [63]. Lower concentrations of MNA could, therefore, lead to increased production of reactive oxygen species, which may promote carcinogenesis [64].

In this thesis, sphingolipid C26:0 concentration is observed to be lower among stage III compared to stage I patients, while another sphingolipid, i.e. sphingolipid C18:0 is observed to be higher among stage IV compared to stage I patients (**Chapter 3**). Sphingolipids play a key role as regulators of cell membrane function [65] and are hypothesized to inhibit the growth of cancer cells [39]. Although this may explain the lower SM C26:0 concentrations among stage III compared to stage I patients, it does not explain the higher concentrations of SM C18:0 among stage IV compared to stage I patients. There may be other factors that are not considered in this thesis, such as for example the tumor size or location, that may play a role in metabolite concentrations across colorectal cancer stages. In addition, in **Chapter 3** and a prior study [38], it is recognized that metabolites do not follow a linear relationship when exploring metabolite concentrations over colorectal cancer stages.

In summary, pathways related to lipogenesis and protein turnover consistently appear important based on the findings of this thesis and earlier studies [46-48, 53, 63] and, therefore, may require additional clinical and nutritional research in relation to the colorectal cancer continuum.

Generalizability of the findings

Colorectal cancer patients originated from multiple countries in the EU and from the US, which increases the generalizability of the presented results in this thesis for the Western colorectal cancer patient population, but some caution must be taken before generalizing the observed findings to the total colorectal cancer patient population worldwide [66]. As a result of the recruitment procedures of some of the cohort studies, some selection might be present. The proportion of stage IV colorectal cancer patients in the presented studies is about half the proportion of stage IV patients observed in the Western colorectal cancer population worldwide [67, 68]. Consequently, results presented might be mainly attributed to stage I-III colorectal cancer.

It is possible that colorectal cancer patients with more complaints or a general poorer health are less prone to participate in a cohort study [69], whereas people who feel relatively well, who are more health-conscious and/or interested in research might be more prone to participate [70, 71]. This may have led to a healthier study population compared to the total colorectal cancer patient population worldwide. It is, therefore, possible that the distribution in exposures and outcomes studied in this thesis might have been narrower than the distribution in colorectal cancer patients worldwide.

In summary, the external validity of the observed findings is believed to be high for Western stage I-III patients but might be fairly low for Western stage IV patients.

Future research perspectives

A clinical perspective

In **Chapters 2** and **3**, the association between circulating metabolites and colorectal cancer occurrence and stage were investigated, respectively. A promising next step in determining metabolites associated with colorectal cancer occurrence would be to identify a pool of metabolites of interest, based on this thesis (**Chapter 2**) and previous studies, and study the identified metabolites in more detail. The metabolites observed with untargeted metabolomics in our and other studies should be taken forward and used in a targeted setting as solely relative differences result from untargeted analysis. It would also be of interest to investigate the association between colorectal cancer patients and colorectal cancer-free individuals using the Biocrates *AbsoluteIDQ*^{TM} p180 Kit used in **Chapters 3** and **4**, however, the kit may not contain all the metabolites identified in untargeted analyses. Since metabolites are also thought to have a heterogeneous nature, as a result of for example different tumor characteristics such as the differentiation grade, tumor size, and tumor location [72], it is also worthwhile to study the association between circulating metabolites and tumor characteristics of colorectal cancer patients in the future.

In addition, despite a plethora of efforts aiming at the discovery of novel blood-based metabolites associated with colorectal cancer [1-10], few reported findings have progressed into examining their predictive properties [73]. Acknowledging the fact that associations between metabolites and colorectal cancer were investigated in this thesis, and not their predictive properties, it is recognized that observed metabolites may have predictive

properties and could, therefore, potentially add to colorectal cancer detection and prognosis practices.

Few studies have investigated the association between circulating metabolites and colorectal cancer stage [3, 12, 13], as was studied in **Chapter 3**. An untargeted approach to gain more insight in which metabolites are associated with colorectal cancer stage might be desired. Although it may be hypothesized that it is not necessary to study stage I and II patients separately, the potential differences between stage II and stage III or IV patients were not studied in this thesis, therefore, it is recommended to compare all individual stages. This thesis consistently hints at the importance of lipid metabolism in the colorectal cancer continuum. If untargeted metabolomics also yields lipids associated with colorectal cancer stage, lipidomics might be promising to further explore lipids specifically. Metabolomics approaches that are not optimized for lipid quantification, i.e. lipidomics, do not safeguard specificity of individual lipids, such as phosphatidylcholines. Lipid species have different physicochemical properties and metabolomics approaches are not able to distinguish fatty acid positional isomers [74]. Using a lipidomics approach could provide more insight in the role of specific lipid species in colorectal cancer progression.

A nutritional perspective

This thesis suggests involvement of lipid and protein metabolism in the colorectal cancer continuum. The intake of dietary fat and protein have not been associated with colorectal cancer occurrence [75], but the association between dietary fat and protein and colorectal cancer progression is currently unknown. Based on the findings of this thesis, it is of interest to determine whether dietary fat and protein may be associated with colorectal cancer progression. A healthier diet is associated with lower circulating phosphatidylcholine concentrations in colorectal cancer patients in this thesis (**Chapter 4**), an association that was also observed in healthy volunteers [15, 16]. It would be of interest to investigate in depth whether dietary intake of fat or protein plays a role in the association between a healthier diet and lower circulating phosphatidylcholine concentrations in colorectal cancer recurrence and survival.

It has been questioned whether a rigorous correction for multiple testing is the best method when investigating metabolomics datasets [76]. The association between diet and metabolite concentrations (**Chapter 4**) might be minor [23, 77] and, therefore, hard to pick up when applying a stringent multiple testing method. Performing multiple statistical tests

increases the probability of a false finding and drawing an invalid conclusion [78]. Applying an additional correction for multiple testing on the observed p -values is crucial to increase the likelihood of a valid conclusion. In **Chapter 4**, it was decided to perform a multiple testing correction as well as to present the top-15 metabolites for the five investigated dietary exposures. It is believed that combining the two approaches might be an appropriate method to report associations between circulating metabolites and dietary exposures, which might also be used in future studies.

Based on this thesis, it also seems that high circulating concentrations of folic acid may have detrimental consequences for colorectal cancer recurrence (**Chapter 5**), which may be a result of the intake of high-dose folic acid-containing dietary supplements and/or high intake of folic acid fortified foods. Therefore, it is crucial to continue to study the association between folate species and colorectal cancer progression and potentially replicate the findings of this thesis. In this thesis, no statistically significant associations with survival were observed but further research is necessary to confirm this observation.

Further research regarding the use of metabolomics in colorectal cancer epidemiology from a nutritional perspective is needed to understand how we can interpret observed associations between nutritional metabolites and health outcomes. The interactions between nutrition, metabolism and health are complicated. It is, thus far, not possible to extrapolate metabolite concentrations solely from a participants' dietary intake or other characteristics. Studies in this thesis show that studying (nutritional) metabolites is promising for gaining insight into the underlying mechanisms of the colorectal cancer continuum, but further research is required to understand the complexity of this relationship.

Consortia of the future

Collaborations with internal and external groups within and outside universities should be appraised to combine forces to create a more efficient use of resources and knowledge since research data is often underused because of a lack of financial resources [79]. Depositing data of a study, once the primary objectives have been achieved, on online archives that embody the FAIR data principles [80], aiming to make scientific data more Findable, Accessible, Interoperable and Reusable in the long-term, would make it more attractive for fellow researchers to reach out and collaborate. In addition to the challenge of data harmonization, using FAIR principles still faces other challenges including finances,

property rights and who to acknowledge in case data would be published. However, it may improve the ability to answer the complex questions related to diet and the colorectal cancer continuum.

The proposed online archive should cover information about study protocols, collected biospecimen, timepoints, conducted or planned measurements on samples, and other available information. Implementing the minimal information checklist, created for nutritional epidemiologic research by Pinart *et al.* [81], might be an excellent tool to screen and identify potential studies to collaborate with. Integrating the minimal information checklist in the online database might help the selection procedure. The checklist contains information regarding the initial study, such as in which country the study was set up, the principle investigator, data sharing policy, study design, used questionnaires, type of biological samples, time point, and which measurements have been conducted in study samples.

Tackling a research question from multiple angles will help to understand the complexity of nutrition and disease. Collaboration among multiple research centers creates multidisciplinary teams, including for example experts in nutritional epidemiology, metabolomics, statistics, and biology. Monitoring expectations of all involved stakeholders is a challenge, and an earlier study has shown that the involvement of more partners may predict fewer project outcomes [82]. Lack of experience in collaboration and stakeholder discussions regarding the scientific lead on a research question can potentially lead to delays in analysis and, as a result, publication of results. Merged PhD projects or more shared first and last authors papers might be needed to make sure multidisciplinary academic teams flourish. A research project manager could be appointed within each research group. Projects managers could oversee current and future analyses within multiple projects and help PhD candidates and principle investigators to navigate through multi-center projects and stakeholder management.

Funding agencies should be aware of the potential benefits of appointing a (part-time) project manager to overview multiple research projects within academic groups. Additional funding streams are necessary to sustain such supporting positions in academia.

Implications

Implications for (future) patients

A lot of confusion is present among patients regarding healthy dietary habits. The increase of social media and so-called experts in nutrition based on personal experiences further reduce clarity. Even if patients turn to their medical doctor for advice regarding diet and lifestyle, they may end up dissatisfied by the competence of medical doctors in giving scientific nutritional recommendations. Most healthcare providers are not trained in giving evidence-based nutrition and lifestyle recommendations for healthy individuals and cancer patients [83]. Healthcare is a multidisciplinary effort in which each stakeholder has its own expertise and, therefore, it is essential that all parties work together. Responsibility regarding the patient's own lifestyle is crucial, and patients might need to ask for advice from a dietician actively. Especially, since it is shown in this thesis and earlier studies that nutrition and lifestyle may play a role in colorectal cancer recurrence and survival and advice by health professionals does help patients to change their diet [84]. Based on this thesis, it is recommended to be careful with the use of high-dose folic acid-containing dietary supplements. It is also advised to discuss with a dietician whether the use of dietary supplements, in general, is needed [83]. This is in line with the current cancer prevention recommendations of the WCRF/AIRC, in which they recommend not to use dietary supplements [75].

Implications for clinical practice

More nutrition and lifestyle training among healthcare providers is needed to create more awareness among clinicians regarding the importance of a healthy lifestyle in disease prevention, treatment and preventing recurrence or reducing quality of life after a cancer diagnosis. However, we should not expect medical specialists to be an expert in nutrition and lifestyle. A solid foundation in understanding the contribution of nutrition and lifestyle and awareness about where to refer a patient to, is the most realistic and time-efficient solution. The findings of this thesis carefully confirm the current WCRF cancer prevention guidelines [75], in which the WCRF/AIRC discourage the use of dietary supplements. At the least, this thesis does not show that the current prevention guidelines need to be changed for colorectal cancer survivors. Clinicians should emphasize the importance of an overall healthy diet, and, when necessary or requested by a patient, refer a patient to a dietician [84, 85].

The findings from this thesis do not have a direct translation to clinical practice but add to the knowledge regarding metabolites involved in the colorectal cancer continuum. In the future, it may provide new insights in the biology of colorectal cancer occurrence and progression. This may finetune colorectal cancer detection and could tailor or change treatment options, to cure colorectal cancer. New insight in the biology of colorectal cancer progression can also help in establishing diet and other lifestyle recommendations to prevent colorectal cancer recurrence. Lastly, the availability of metabolites associated with colorectal cancer progression may also help clinicians to monitor current and cured colorectal cancer patients. Good connections between research groups and clinicians remain essential to continue research among colorectal cancer patients and make findings increasingly generalizable and clinically applicable worldwide.

Overall conclusions

In conclusion, results presented in this thesis show that mainly circulating amino acids and lipid species are associated with colorectal cancer occurrence and stage III-IV compared to stage I colorectal cancer. No differences are observed when comparing stage II to stage I colorectal cancer patients. Adherence to a healthier diet is associated with lower phosphatidylcholine concentrations in colorectal cancer patients. Higher circulating concentrations of unmetabolized folic acid are associated with more colorectal cancer recurrences but no associations are reported for colorectal cancer survival. Circulating concentrations of folate and folate catabolites are not associated with colorectal cancer recurrence and survival.

Investigating metabolite concentrations across the colorectal cancer continuum is a helpful methodological approach from a clinical and nutritional perspective. Based on the observations of this thesis, it can be hypothesized that amino acid and lipid metabolism may play important roles in the colorectal cancer continuum and further research in this area is necessary. In addition, it was shown that higher compared to lower concentrations of circulating folic acid is associated with an increased risk of colorectal cancer recurrence. Awareness of the potential harmful consequences of high intakes of folic acid-containing dietary supplements and/or folic acid fortified food products may be important. This observation supports the 2018 cancer prevention recommendations of the WCRF/AIRC, in which it is recommended not to use dietary supplements [75].

The interplay between metabolites, nutrition and colorectal cancer progression requires more studies to evaluate the role of metabolites in the underlying biology of nutrition in the colorectal cancer continuum. The optimal clinical and nutritional approach to further study metabolites across the colorectal cancer continuum would be to combine untargeted and targeted approaches, including discovery-replication designs to allow for robust findings [30] and ensure generalizability to the colorectal cancer population worldwide. Standardization across studies is important, i.e. study samples should be collected in a standardized way, preferably all fasted, to be able to combine datasets while also considering demographic and nutritional characteristics of the study population, study designs, colorectal cancer stages and tumor characteristics. National and international collaborations remain crucial to be able to unravel the clinical and nutritional complexity of the colorectal cancer continuum.

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Summary

Colorectal cancer is one of the most prevalent cancers worldwide. Genetic, environmental, nutritional, and other lifestyle factors play a large role in the development of colorectal cancer, but the role in colorectal cancer recurrence and survival is largely unknown. Measuring metabolites, i.e. molecules present in biospecimens, such as blood and tissue samples, can help to unravel the underlying biology of the colorectal cancer continuum. The colorectal cancer continuum reflects the process in which normal cells progress into colorectal cancer cells. Therefore, the overall aim of this thesis is to better understand the association between circulating metabolites and the colorectal cancer continuum from a clinical and nutritional perspective in two international consortia.

In **Chapter 2**, the association between plasma metabolites and colorectal cancer occurrence is studied using an untargeted metabolomics approach. Data of 268 stage I-IV colorectal cancer patients and 353 colorectal cancer-free individuals of an international consortium are included. Data are split into an independent discovery and replication set. After replication, 28 molecules are associated with colorectal cancer, including 15 molecules that could be identified as known metabolites. Levels of taurine, hypoxanthine, lysophosphatidylethanolamines (LysoPEs) (20:4) and (22:6) are shown to be higher among colorectal cancer patients compared to the colorectal cancer-free individuals. The opposite is observed for valine, leucine, bilirubin, 1-methylnicotinamide, and seven phosphatidylcholines (LysoPCs), namely LysoPC(15:0), LysoPC(16:0), LysoPC(16:0) isomer, LysoPC(P-16:0), LysoPC(16:1), LysoPC(17:0), and LysoPC(18:0), which show lower levels among colorectal cancer patients compared to colorectal cancer-free individuals.

The association between plasma metabolites at diagnosis and colorectal cancer stage is investigated using a targeted metabolomics approach in **Chapter 3**. In total, 744 patients with stage I-IV colorectal cancer from an international consortium are included in the analysis. Sphingomyelin (SM) C26:0 show lower concentrations among patients with stage III compared to stage I colorectal cancer. SM C18:0 and phosphatidylcholine diacyl (PC aa) C32:0 concentrations are higher, whereas citrulline, histidine, PC aa C34:4, PC acyl-alkyl (ae) C40:1 and LysoPC a C16:0 and C17:0 are lower among stage IV compared to stage I colorectal cancer patients. No differences in plasma metabolite concentrations are observed between stage II and stage I colorectal cancer patients.

In **Chapter 4**, two diet quality indices and three dietary patterns, and their associations with plasma metabolites in colorectal cancer patients are shown. Plasma

metabolites of 195 stage I-IV colorectal cancer patients are quantified using a targeted metabolomics approach. Two widely acknowledged diet quality indices are used, the 2018 dietary cancer prevention recommendations of the World Cancer Research Fund (WCRF), i.e. the WCRF dietary score, and the Dutch Healthy Diet Index (DHD15-index), based on the Dutch dietary guidelines of 2015. A higher intake of a healthier diet is reflected by a higher WCRF score and DHD15-index. The three dietary patterns are identified based on the reported habitual dietary intake of the colorectal cancer patients by Food Frequency Questionnaire at cancer diagnosis, i.e. a Western, Carnivore and Prudent pattern. The Western and Carnivore pattern reflect high intake of items usually present in an unhealthier diet. The Western dietary pattern is characterized by a high intake of snacks, savory sauces and spreads, refined grains, pizza, high and medium-fat dairy, nuts and seeds, beer, and hard fats, and a low intake of whole grain products and potatoes. The Carnivore pattern is characterized by a high intake of red and processed meat, poultry, fish, eggs, and potatoes, and a low intake of soy and vegetarian products, and medium and high-fat dairy. The Prudent pattern reflects a healthy diet, with high consumptions of vegetables, fruits, fish, nuts and seeds, and low-fat dairy and low intakes of pastry and biscuits, and beer. Better adherence to healthier diets is associated with lower concentrations of plasma phosphatidylcholines (**Chapter 4**). More specifically, patients with better adherence to the WCRF dietary score, DHD guidelines and higher consumptions of the Prudent pattern show lower concentrations of plasma phosphatidylcholines. Contrary, patients with higher intakes of the Western and Carnivore patterns show higher concentrations of plasma phosphatidylcholines.

Chapter 5 describes the associations between circulating concentrations of folate species at diagnosis and colorectal cancer recurrence and survival. The folate species folate, folic acid, and folate catabolites p-aminobenzoylglutamate (pABG) and p-acetamidobenzoylglutamate (apABG) are quantified using a targeted metabolomics approach in blood samples of 2024 stage I-III colorectal cancer patients within an international consortium. Circulating concentrations of folate, pABG and apABG are not associated with colorectal cancer recurrence and survival. Higher compared to lower concentrations of folic acid are associated with an increased risk of recurrences, but folic acid concentrations are not associated with colorectal cancer survival.

The findings of this thesis suggest that amino acid and lipid metabolism may play important roles in the colorectal cancer continuum. In addition, higher circulating concentrations of folic acid may be associated with an increased risk of colorectal cancer

recurrence. Based on the observations in this thesis, investigating metabolite concentrations across the colorectal cancer continuum may provide important leads to further study the underlying biology of colorectal cancer development and progression.

This thesis also showed that national and international collaborations are valuable because of the availability of data from colorectal cancer patients from several countries and the availability of a wide variability in exposures and outcomes. Future national and international collaborations are, therefore, encouraged to further study the underlying biology of the complex interplay between metabolites, nutrition, and the colorectal cancer continuum from a clinical and nutritional perspective.

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About the author

Anne Johanna Maria Renée Geijsen was born on June 6th, 1990 in the beautiful Maastricht, the Netherlands. In 2008, she completed secondary school at the Provinciale School in Voeren, Belgium. She followed the BSc Voeding & Gezondheid at Wageningen University & Research, the Netherlands. Afterwards, she worked for a year as a Research Assistant for the Maastricht Studie at Maastricht University, the Netherlands. She completed her MSc degree in Nutrition & Health at Wageningen University & Research in 2015. During her master, she wrote two theses. One thesis was focused on studying the effect of intense exercise on gastrointestinal permeability among healthy male athletes as part of the Protégé study. Her second minor thesis investigated the effect of trans fatty acids on cellular metabolism, Angptl4 expression and inflammation in human macrophages. She completed her MSc internship at Nutricia Research in Utrecht, the Netherlands. As part of the GRINTA study, she studied the potential of saliva as a biospecimen to measure various inflammatory markers related to gastrointestinal integrity of healthy, recreational male athletes. In 2015, Anne started as a PhD candidate at the Human Nutrition and Health division of Wageningen University. She studied (nutritional) metabolites in relation to colorectal cancer aetiology and progression. The projects were embedded in two international consortia, the MetaboCCC and FOCUS consortia. The results of these projects are described in this thesis. She is currently working as a project manager at the Medical Innovation and Technology expert Center (MITeC) at Radboud University, Nijmegen, the Netherlands.

Anne is interested in various aspects of life sciences, including nutrition, gut and mental health, and technological innovations towards a healthier and more sustainable life for current and future generations. She has too many hobbies, including cooking, reading, power yoga, trying out new creative activities, triathlons, bouldering, volunteering at the local vineyard in Wageningen, and enjoying time in nature. She hiked the Fjällraven Classic in Sweden, the Landmannalaugar trail in Iceland, explored the Annapurna region in Nepal, and Colorado in the USA, and recently went on a solo trip to Chile and Argentina. Closer to home, she is currently enjoying the incredible nature around her cottage near Wageningen.

Overview of completed training activities

Discipline specific activities	Organiser and location	Year
Basiscursus oncologie	NVvO, Ellecom, NL	2016
Introduction to Nutritional Metabolomics	Copenhagen University, Denmark	2016
EMBO Practical Course on Metabolomics	EMBL-EBI, Cambridge, UK	2017
Swedish Metabolomics Conference	Chalmers University, Goteborg, Sweden	2017
BBMRI Workshop	BBMRI, Leiden, NL	2016
Chemometrics Course	Wageningen University, NL	2017
Metabolomics Seminar and Networking Event	Maastricht University, NL	2018
Masterclass "Nutrition and Cancer: from Bench to Bed to Behaviour"	VLAG, Wageningen, NL	2019
General courses and activities		
VLAG PhD week	VLAG, Wageningen, NL	2015
Project and Time Management	WSG, Wageningen, NL	2017
Effective Behaviour in your Professional Surroundings	WSG, Wageningen, NL	2017
PhD Peer Consultation	WSG, Wageningen, NL	2017
Teaching and Supervising thesis students	ESD, Wageningen, NL	2018
Brain-friendly Working and Writing	WSG, Wageningen, NL	2018
Career Perspectives	WSG, Wageningen, NL	2019
Diet and Microbes	Helsinki University, Levi, Finland	2020
Optional courses and activities		
Preparation of PhD research proposal	WUR, Wageningen, NL	2015
Organisation of the PhD tour 2017	WUR, Wageningen, NL	2016-2017
PhD study tour to the UK		2017
Staff seminars and chair group meetings	WUR, Wageningen, NL	2015-2019
MSc Concepts and Methods in Epidemiology	WUR, Wageningen, NL	2015
Monthly webinars on metabolomics-related topics (Metabolomics Society and NuGo)		2015-2019

COLOPHON

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