Cardiovascular Risk Factors Associated With Blood Metabolite Concentrations and Their Alterations During a 4-Year Period in a Population-Based Cohort

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Background—The effects of lifestyle risk factors considered collectively on the human metabolism are to date unknown. We aim to investigate the association of these risk factors with metabolites and their changes during 4 years.

Methods and Results—One hundred and sixty-three metabolites were measured in serum samples with the AbsoluteIDQ kit p150 (Biocrates) following a targeted metabolomics approach, in a population-based cohort of 1030 individuals, aged 45 to 83 years at baseline. We evaluated associations between metabolite concentrations (28 acylcarnitines, 14 amino acids, 9 lysophosphocholines, 72 phosphocholines, 10 sphingomyelins and sum of hexoses) and 5 lifestyle risk factors (body mass index [BMI], alcohol consumption, smoking, diet, and exercise). Multilevel or simple linear regression modeling adjusted for relevant covariates was used for the evaluation of cross-sectional or longitudinal associations, respectively; multiple testing correction was based on false discovery rate. BMI, alcohol consumption, and smoking were associated with lipid metabolism (reduced lyso- and acyl-alkyl-phosphatidylcholines and increased diacylphosphatidylcholines concentrations). Smoking showed positive associations with acylcarnitines, and BMI correlated inversely with nonessential amino acids. Fewer metabolites showed relative changes that were associated with baseline risk factors: increases in 5 different acyl-alkyl phosphatidylcholines were associated with lower alcohol consumption and BMI and with a healthier diet. Increased levels of tyrosine were associated with BMI. Sex-specific effects of smoking and BMI were found specifically related to acylcarnitine metabolism: in women higher BMI and in men more pack-years were associated with increases in acylcarnitines.

Conclusions—This study showed sex-specific effects of lifestyle risks factors on human metabolism and highlighted their long-term metabolic consequences. **(*Circ Cardiovasc Genet*. 2016;9:487-494. DOI: 10.1161/CIRCGENETICS.116.001444.)**

Key Words: acylcarnitines ■ amino acids ■ cardiovascular disease ■ lifestyle ■ lipids ■ metabolomics

Cardiovascular disease (CVD) is a leading cause of mortality, accounting for >30% of deaths worldwide.[1] There are several modifiable (ie, obesity, smoking, alcohol, physical activity, and diet) and also unmodifiable risk factors (ie, sex and age) associated with CVD; however, the pathophysiological mechanisms of the association between risk factors and disease are not yet fully understood.

Clinical Perspective on p 494

Metabolomics aims to quantify small molecules present in a biological system at a specific time point. This snapshot provides an indication of the current metabolic state of the whole organism at a given stage of life. Differences in metabolic profiles can be because of pathological stimuli, environmental impact, or normal physiological variations. Several studies have shown that metabolomics can identify distinct metabolic patterns in individuals affected by pathological conditions, that is, atherosclerosis, diabetes mellitus, or cardiovascular disorders.[2–5]

The effects of several classic modifiable CVD risk factors on metabolism have already been studied in different population-based cohorts. In particular, alcohol intake, obesity, diet patterns, and smoking were shown to be associated

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with alterations in lipid metabolism. Additionally, obesity and smoking were associated with alterations in amino acids metabolites.[6–9] All these studies compared individuals with or without a determined risk factor at a given time point, cross-sectionally. However, the long-term effects of these risk factors on the human metabolism are to date unknown, that is, are they associated with changes in metabolite concentrations? Moreover, in previous studies, modifiable risk factors have been considered independently of each other, although it is well established that together they form a risk profile with a high predictive value for CVD.[10] A further not yet completely studied question is whether there are sex-specific differences in the effects of modifiable risk factors on metabolism. Hence, we aim to determine whether modifiable risk factors are associated with differences in metabolite concentrations and whether risk factors predict changes over time (4 years) in relevant metabolites in a healthy population (without CVDs or diabetes mellitus). Furthermore, sex-specific associations will be studied.

Methods

Study Population

The CARLA (Cardiovascular disease, Living and Ageing in Halle) study is a population-based cohort study in an elderly population of the city of Halle/Saale in eastern Germany. Study design and methods were described in detail elsewhere.[11] In brief, subjects were recruited randomly from the population registry in a multistage process. At baseline, 1779 subjects (46% women) aged 45 to 83 years were examined between July 2002 and January 2006. After a mean of 4-year follow-up (SD=0.3), 1436 subjects (45% women) took part in the first follow-up examination (response rate 92%). The current analysis included a total of 1030 participants, who were at both time points free of severe CVD (myocardial infarction, self-reported coronary artery bypass graft, self-reported percutaneous transluminal coronary angioplasty, self-reported physician-diagnosed stroke, and carotid surgery) and of diabetes mellitus (defined by self-report and medication) and had metabolite measurements at both time points (see Figure I in the Data Supplement for flowchart of the study). No differences were seen in sex distribution among participants excluded and included, but those excluded tended to be older. The CARLA study was performed in accordance with the Declaration of Helsinki. All participants gave their written informed consent. The study was approved by the local ethics commission of the Medical Faculty of the Martin-Luther University Halle-Wittenberg.

Metabolomics Measurements

For this study, nonfasting blood serum samples of the study participants were analyzed using a targeted metabolomics approach. Blood samples were taken after a supine rest of 30 minutes. After a 10-minute centrifugation (20 °C; 1500 rpm), the serum was collected, and after a clotting time of 30 minutes, deep frozen to −80 °C on the same day and stored until analysis of the metabolites.

Metabolite quantification was performed for both time points together and randomly in the Genome Analysis Center at the Helmholtz Zentrum München. Out of 10 µL blood serum, we quantified simultaneously a panel of 163 metabolites that include free carnitine, 40 acylcarnitines (acylC), 14 amino acids (AA), hexoses (sum of hexoses), 92 glycerophospholipids (15 lysophosphatidylcholines [lysoPC] and 77 phosphatidylcholines [PC]), and 15 sphingolipids (SM) using flow injection analysis-tandem mass spectrometry and the Absolute*IDQ* p150 kit (Biocrates Life Sciences AG, Innsbruck, Austria). The assay is performed on a double-filter 96-well plate containing isotope-labeled internal standards, which were taken as reference for metabolite quantification. The procedures for sample preparation and mass spectrometric measurements as well as the metabolite nomenclature have been described in detail previously.[12] The method has been successfully applied in multiple academic and industrial settings. For a full list of all quality-controlled metabolites, see Table I in the Data Supplement.

Risk Factors Assessment

The examinations at baseline and follow-up investigations included a standardized computer-assisted personal interview, self-administered questionnaires, a medical examination by trained personnel, and drawing of a nonfasting venous blood sample. The standardized, computer-assisted interview collected information on sociodemographic and socioeconomic variables, psychological and biomedical factors, medical history, and use of medication within the preceding 7 days. Medication was coded according to the Anatomical Therapeutic Chemical Classification System.

Being physically active was defined with the Baecke questionnaire, which addresses different types of physical activity, including unstructured activities performed in leisure time and transportation to work.[13] Information on smoking habits involved questions on past and current smoking status; duration of smoking; and quantity of tobacco products smoked per day. We used a continuous measure of smoking: pack-years of tobacco products ever smoked. Self-reported usual consumption of alcohol in grams per day was calculated from the answer to the questions "How much beer (in units of 0.5 L)…," "How much wine or champagne (in units of 0.2 L)…," and "How many glasses of spirits (2 cL/glass)... do you usually drink during a week?" The body mass index (BMI; kg/m^2) was calculated using standardized weight and height measurements. Dietary patterns were determined based on the Food Frequency Questionnaire.[14] Participants were asked how often (on average) they eat the following food items: whole meal products, vegetables, chocolate, meat, etc. These categories were accumulated into a food frequency index reflecting dietary quality within a range of 0 (unhealthier dietary pattern) to 30 (healthier dietary pattern).

Statistical Analysis

The statistical analysis system SAS version 9.3 (SAS Institute, Inc; Cary, NC) was used for the statistical analysis and Circos software[15] to generate the plots. Metabolite concentrations were log transformed because in most cases the log-transformed concentrations were closer to a normal distribution than the untransformed values. The preselected modifiable risk factors at baseline were as follows: alcohol use (gram per day), pack-years of tobacco, physical activity, BMI, and dietary patterns. Three risk factors (alcohol, pack-years, and sport score) had to be logarithmized as the regression residuals showed a non-normal distribution in a QQ-plot that could influence the results. Confounders were age, sex, fasting time, month of drawing, medication use (yes/no) according to Anatomical Therapeutic Chemical Classification System code (13 medication groups), number of diseases (Charlson Comorbidity Index[16]), systolic and diastolic blood pressure, triglycerides, glucose and high-density lipoprotein cholesterol level, and typical daily consumption of tea or coffee.

Cross-Sectional Associations Between Risk Factors and Metabolite Levels

For the cross-sectional associations, we used linear regression analysis to determine the relationship between each risk factor and each metabolite, adjusted for confounders. To control for the effect of multiple testing (134 metabolites for 5 different risk factors), we used the false discovery rate using the Benjamini–Hochberg method[17] with an false discovery rate <0.05.

Furthermore, to check which subclasses of metabolites were associated to each risk factor, analyses were repeated by metabolite groups (AAs; short-chain, medium-chain, and long-chain acylC; PC diacyl; PC acyl-alkyl; SM; and lysoPC).

Associations of Risk Factors With Relative Changes in Metabolite Levels

Longitudinal analyses were done with linear regression models to determine the associations between risk factors at baseline and relative changes in metabolite concentrations during 4 years ([follow-up

value−baseline value]/baseline value). Several previous publications have shown different effects for risk factors on metabolites for men and women.[7,18] Therefore, we included interaction terms between risk factor and sex in those models where the interaction was significant (P<0.05). Correction for multiple testing was done via false discovery rate estimation. To ensure that the identified associations were not driven by study participants with major changes in their risk factors, we performed a sensitivity analysis of participants with stable risk factor exposure over time. These analysis were performed for participants who had no or minimal changes in risk factors between baseline and follow-up (n=979). This was defined as changes in BMI <20%; changes in diet pattern <20%; changes in alcohol consumption <20%; or in case of sport, being either physically active or inactive at both time points; or smoking, being either a smoker or nonsmoker at both time points.

Associations Between Metabolite Groups and Clinical Conditions

To investigate the clinical relevance of the observed associations, further logistic and linear regression analyses were done to explore the relationship between metabolite groups and clinical end points (ie, metabolic syndrome, insulin resistance, inflammation, and oxidative stress). Metabolic syndrome was defined as having >3 of the following symptoms[19]: elevated waist circumference (102 cm for men and 88 cm for women); elevated triglycerides (1.7 mmol/L); reduced high-density lipoprotein cholesterol (1.0 mmol/L for men and 1.3 mmol/L for women); elevated blood pressure (systolic ≥130 and/or diastolic ≥85 mm Hg); and elevated fasting glucose (≥5.6 mmol/L). Insulin resistance was defined by elevated nonfasting glucose levels, inflammation as elevated C-reactive protein levels, and oxidative stress as decreased levels of RAGE (receptor for advanced glycation endproducts). All models were adjusted for BMI, sex, and age.

Results

Description of the CARLA Cohort

The characteristics of healthy participants who took part in both assessments (baseline and follow-up after 4 years) are shown in Table 1. Participants were on average aged 62 years at baseline, and almost half of the participants (48%) were women. The number of current smokers decreased slightly with time, as well as the alcohol consumption, whereas the percentage of physically active participants increased. BMI and diet score remained rather stable over time.

Cross-Sectional Associations Between Risk Factors and Metabolite Levels

In the cross-sectional analysis, baseline and follow-up cohorts were merged for a higher statistical power (N=2060). The metabolites associated with each classical risk factor are shown in Figure and Table II in the Data Supplement (Table III in the Data Supplement additionally shows the association of groups of metabolites with each classical risk factor). BMI was negatively associated with 4 lysoPC and 7 acyl-alkyl PC; that is, greater BMI was associated with lower concentrations of those metabolites. On the contrary, associations between BMI and diacyl PC (2 long-chain) were positive. Additionally, a sex-specific, positive association with carnitine, acetylcarnitine, and hydroxyacylC was observed for women with higher BMI. Tyrosine was positively associated with BMI, whereas glutamine and glycine in the total population and serine in men were negatively associated with BMI. A healthier diet was associated with higher blood concentrations of a long-chain diacyl PC. Alcohol consumption was negatively associated with a lysoPC, several acyl-alkyl PC, and 3 hydroxy-SM and positively with 2 diacyl PC and a lysoPC. For men, we also found associations with an acylC. Pack-years of tobacco were positively associated with 7 acylC predominantly in men, and negatively associated with a lyso PC. Seven diacyl PCs were positively associated with tobacco in the total population and in men only; 6 acyl-alkyl PCs were negatively associated with tobacco in women. Finally, regular physical activity was negatively associated with arginine in men.

Associations of Risk Factors With Relative Changes in Metabolite Levels

In the next step, we investigated whether baseline risk factors associate with concentration changes from baseline to follow-up for individual metabolites. Results are summarized in Table 2 and Table IV in the Data Supplement. BMI at baseline was associated with an increase in tyrosine levels during the 4-year follow-up and for women with a reduction in an acyl-alkyl PC (PC ae C34:0). Higher alcohol consumption at baseline was associated with a decrease in levels of 4 acyl-alkyl PC (PC ae C34:2, C36:2, C38:3, and C40:6) during the

Table 1. Sex-Stratified Characteristics of the Study Sample

	Baseline		Follow-Up		Change From Baseline to Follow-Up	
	Men (n=534)	Women (n=496)	Men (n=534)	Women (n=496)	Men (n=534)	Women (n=496)
Age, y, mean (SD)	61.9 (9.8)	61.4 (9.0)	65.9 (9.7)	65.5 (9.0)	…	…
Smoker						
Current, n (%)*	133 (24.9)	72 (14.5)	113 (21.2)	62 (12.5)	32 (6.0)	20 (4.0)
Pack-years (for current smokers), mean (SD)	13.3 (15.1)	4.0 (8.6)	14.0 (16.2)	4.4 (9.3)	0.7 (2.0)	0.3 (1.0)
Sport						
Yes, n (%)*	188 (35.2)	233 (47.0)	232 (43.5)	274 (55.0)	116 (21.7)	131 (26.4)
Alcohol, g/d, last year, mean (SD)	19.4 (18.8)	4.9 (7.9)	16.7 (16.9)	4.5 (7.9)	−2.8 (13.3)	−0.4 (5.0)
BMI, kg/m^2, mean (SD)	27.5 (3.5)	27.5 (4.9)	27.7 (3.7)	27.9 (5.2)	0.3 (1.2)	0.4 (1.6)
Diet, FFI (0=unhealthy to 30=healthy), mean (SD)	14.0 (3.2)	16.2 (3.2)	14.5 (3.1)	16.7 (3.0)	0.4 (2.8)	0.4 (2.8)

BMI indicates body mass index; and FFI, food frequency index.
*Changes occurred in both directions from absence to presence and vice versa.

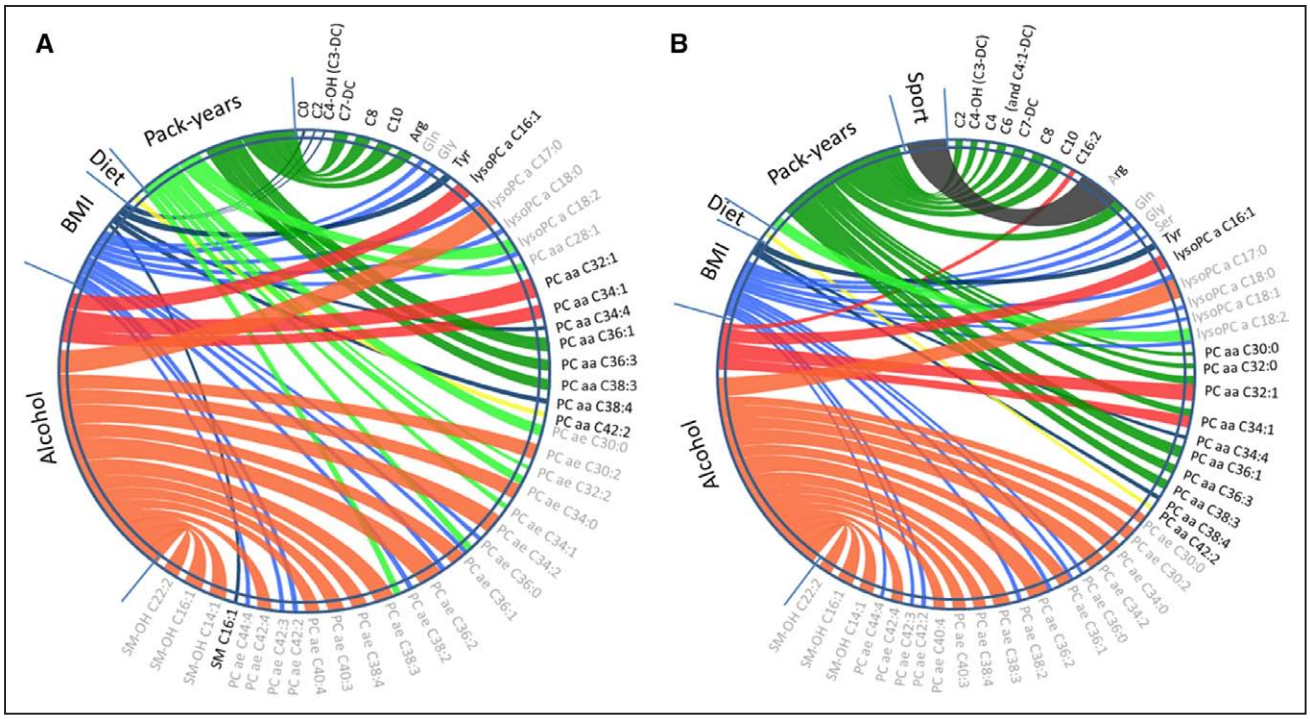

Figure. Circos plot of the association between risk factors (separated into positive (dark) and negative (light) associations) on the left side and individual metabolites on the right side for (**A**) women and (**B**) men. Width of curves indicates strength of the association (β effect). AA indicates amino acid; BMI, body mass index; PC, phosphatidylcholine; and SM, sphingolipid.

follow-up period. Diet was associated with an increase in levels of an acyl-alkyl PC (PC ae C40:6). Pack-years of tobacco were associated with an increase in levels of PC aa C32:1.

Associations Between Metabolite Groups and Clinical Conditions

Baseline AA levels were predictive of chronic health conditions. Logistic regression analyses showed that all 3 glucogenic amino acids (Gtn, Gly, and Ser) at baseline were associated with metabolic syndrome at follow-up. Higher concentrations of glutamine, glycine, and serine had a protective effect on the development of metabolic syndrome 4 years later (odds ratio for gtn=0.82 [95% confidence interval, 0.69–0.98; P<0.04] for men only; odds ratio for gly=0.59 [95% confidence interval=0.50–0.70; P<0.0001]; and odds ratio for ser=0.75 [95% confidence interval=0.65–0.86; P<0.0001] in the total population).

Lipid levels were associated cross-sectionally and longitudinally with insulin resistance and inflammation (see Table V in the Data Supplement). At baseline, higher diacyl PC (β=0.001; SE=0.0003; P<0.01) and lower acyl-alkyl levels (β=−0.001; SE=0.0003; P<0.01) but not lysoPC levels (β=−0.0003; SE=0.0007; P=0.64) predicted higher glucose levels at follow-up. Similar results were obtained for cross-sectional associations. Higher inflammation at follow-up, as measured by C-reactive protein levels, was also associated with higher diacyl PC levels at baseline (β=0.005; SE=0.002; P<0.02) and with lower lysoPC levels at baseline (β=−0.02; SE=0.005; P<0.0001) but not with acyl-alkyl PC levels (β=−0.002; SE=0.002; P=0.37). C-reactive protein levels were not associated to diacyl PC in cross-sectional analysis; results for acyl-alkyl and lysoPC were similar also for cross-sectional associations. Additionally, in cross-sectional analysis, lower levels of diacyl PC and lysoPC and higher levels of acyl-alkyl PC were associated with higher levels of RAGE (β=−3.38; SE=0.98; P<0.001, β=−4.31; SE=1.96; P<0.03, and β=2.70; SE=0.90; P<0.003, respectively).

Discussion

In our study, we investigated the effects of a set of modifiable CVD risk factors (BMI, diet, alcohol and tobacco consumption, and physical activity) on blood metabolite concentrations and their changes during 4 years for 534 men and 496 women from the population-based CARLA cohort. In our cross-sectional analyses, we replicated numerous findings from previous studies, where it was shown that metabolite profiles largely reflect altered lipid metabolism in case of obesity, alcohol consumption, and smoking.[8,20] The number of metabolites, for which we observed a significant concentration change associated with these baseline risk factors after a period of 4 years, was much smaller compared with the results of the cross-sectional analysis, which considered risk factors and metabolite concentrations measured at the same time point. This was expected as the metabolic profile represents a momentary picture reflecting the physiological state of a system at a given time. However, interestingly, our longitudinal analyses indicated long-term effects of modifiable CVD risk factors on many of the same metabolites that contribute to chronic disease pathologies. A further interesting finding was the sex specificity of some of these associations. Below, we discuss our results according to metabolite classes across the different risk factors.

Acylcarnitines

An unhealthy lifestyle, defined as higher BMI, greater alcohol consumption, and greater cigarette consumption, was

Table 2. Longitudinal Associations Between Baseline Lifestyle Risk Factors and Relative Changes in Metabolite Values

		Longitudinal		Cross-Sectional	
Risk Factor	Metabolite	β (SE)	*P* (FDR)	β (SE)	*P*
BMI					
	Tyr	0.03 (0.01)	0.0003 (0.03)	0.09 (0.02)	<0.0001
	PC ae C34:0	–0.06 (0.01)*	<0.0001 (0.01)*	–0.03 (0.02)	0.08
Alcohol					
	PC ae C34:2	–0.09 (0.02)	0.0003 (0.03)	–0.23 (0.05)	<0.0001
	PC ae C36:2	–0.11 (0.02)	<0.0001 (0.01)	–0.27 (0.05)	<0.0001
	PC ae C38:3	–0.09 (0.02)	<0.0001 (0.02)	–0.22 (0.05)	<0.0001
	PC ae C40:6	–0.09 (0.02)	0.0002 (0.02)	–0.11 (0.05)	0.02
Diet					
	PC ae C40:6	0.04 (0.01)	0.0001 (0.02)	0.02 (0.02)	0.21
Pack-years					
	PC aa C32:1	0.09 (0.02)	<0.0001 (0.01)	0.21 (0.04)	<0.0001

Analyses were adjusted for sex (in total analysis), age, fasting time, batch, medication use according to ATC code, systolic blood pressure, diastolic blood pressure, HDL cholesterol, triglycerides, glucose levels, and consumption of tea or coffee. ATC indicates Anatomical Therapeutic Chemical Classification System; BMI, body mass index; FDR, false discovery rate; and HDL, high-density lipoprotein.

*Significant associations only for women.

positively associated with concentration levels of mostly short- and medium-chain acylC. AcylC are related to β-oxidation of fatty acids or AA metabolism and are markers of mitochondrial dysfunction.[21]Several of them, that is, C2, C6, and C8, have been proposed to be useful indicators of metabolic changes, particularly related to disease states.[22] To check which subclasses of acylC were associated to the phenotypes, analyses were repeated by metabolite groups (Table III in the Data Supplement). Short-chain acylC and only nominally medium-chain acylC were positively associated with BMI and pack-years. The long-term effects of smoking were not associated with changes in the concentrations of any acylC during 4 years. It has been previously shown that most of these effects can be reversed by smoking cessation.[9]

Amino Acids

This class of metabolites was mostly associated with BMI. Additional associations were seen between physical activity and lower concentration of Arg, whereas smoking was associated with a higher concentration of Arg. A former study on the metabolite profile of coronary artery disease showed an increase in urea cycle–related metabolites, including arginine,[23] which we also identified in our study as smoking-related metabolite (positively) and sport-related metabolite (negatively).

The association pattern between BMI and Tyr (increase) was also in line with an incipient insulin resistance associated with higher BMI.[24] Already in 1969, it was reported that obesity was associated with elevated levels of 2 aromatic AA (tyrosine and phenylalanine) and branched-chain AA (valine, leucine, and isoleucine) and decreased levels of glycine. Moreover, the concentration of those elevated AA in obesity correlated with serum insulin levels.[25] Further studies have reported that glutamine, serine, and glycine are significantly decreased in obese individuals when compared with lean controls.[26,27] Although we replicated previous findings for tyrosine, glutamine, serine, and glycine, we observed only nominally significant associations between branched-chain AA and obesity (valine: β=0.04; SE=0.02 and leucine: β=0.03; SE=0.02), which did not remain significant after multiple test correction. A possible reason is the nonfasting state of the study population. Branched-chain AAs are strongly influenced by fasting state.[28] In the longitudinal study, we found evidence for a persistent positive association between BMI and tyrosine.

The relationship between obesity and glucogenic amino acids (glutamine, glycine, and serine for men) can be explained by their role in glucose metabolism.[29] Total body glutamine is reduced in catabolic states such as trauma and infection.[30] The consistent associations observed between glutamine-related metabolites and metabolic traits have led to the hypothesis that glutamine-cycling pathways are prominently involved in the development of metabolic syndrome.[31] We could see in our population that glutamine (in men), glycine, and serine at baseline were protective factors for the development of metabolic syndrome. We found significantly reduced glutamine in obese compared with normal-weight participants during a 4-year time span. To our knowledge, only one study on children has shown this obesity-related reduction of glutamine.[32]

Lipids

Our results showed that BMI, alcohol consumption, and smoking were associated with lipid metabolism; in particular, unhealthier lifestyle was associated with lower lysoPCs and acyl-alkyl PCs and higher diacyl PCs. Moreover, for smoking, a sex-specific pattern could be observed. These results are in accordance with previous studies that also showed that obese participants had higher concentrations of diacyl PC at the expense of acyl-alkyl

PC and lysoPC. It is hypothesized that this altered lipid profile in obese individuals facilitates inflammation and insulin resistance. For example, Szymańska et al[33] showed a robust decrease of acyl-alkyl PC and lysoPC (catabolic products of PCs) in a healthy population with central obesity. Whereas Pietiläinen et al[34] showed in a twin study that the total cholesterol concentration and insulin resistance correlated negatively with acyl-alkyl PC in obese individuals compared with their nonobese twins. This observed decrease in acyl-alkyl PCs in obese participants may reflect less lipid remodeling.[35] Because no further decreases or increases in lipid concentrations of these lipids could be seen during 4-year time point, the impact of BMI on lipid metabolism as observed in our cross-sectional analysis seems to reflect an instantaneous rather than a long-term effect. We showed that participants with higher diacyl PC or lower acyl-alkyl PC levels had potential insulin resistance. Also, participants with lower lysoPC levels had higher inflammation levels. This pattern is even clearer for their predictive value, where diacyl PC and acyl-alkyl have opposite roles in the prediction of insulin resistance, and similarly, diacyl PC and lysoPC have inverse roles in the prediction of inflammation levels.

Alcohol consumption also vastly affected lipid metabolism, as previously reported.[7] In agreement with Jaremek et al, we also observed alcohol consumption to be associated with a decrease in lysoPC a C17:0 but an increase in lysoPC a C16:1, an increase in PC aa C32:1 and PC aa C34:1, and a decrease in several acyl-alkyl PCs and SM. The concentration and composition of plasma lysoPCs is the result of several pathways; they can originate from hepatic secretion or be directly synthesized from lipoprotein-PC.[36] We showed that higher levels of lysoPC were associated with lower levels of RAGEs, which is a biomarker for an elevated level of oxidative stress in the cells. There are several ways in which ethanol can induce oxidative stress: via increased malondialdehyde formation as demonstrated by incubated liver preparations; through the absorption of conjugated dienes in mitochondrial and microsomal lipids; and by the decrease in the most unsaturated fatty acids in liver cell membranes.[37] The decrease in lysoPC C17:0 could be the result of a confounding effect. It has been shown that this metabolite is associated with milk consumption, and in our sample, milk consumption is negatively associated with alcohol (r=–0.14; P<0.0001). Similar to results for BMI, lower acyl-alkyl PC levels for higher alcohol consumption might indicate less lipid remodeling in the membranes. But in contrast to the results for BMI, lower acyl-alkyl PC levels are not combined with higher diacyl PC levels. Alcohol showed a longitudinal effect on several acyl-alkyl PC. Those metabolites have been previously negatively associated with moderate/severe chronic alcohol consumption.[7] The underlying mechanism for lower SM concentrations with higher alcohol consumption could be attributed to the activity of the acid sphingomyelinase, an enzyme responsible for the catabolism of sphingomyelins.[38] Several studies indicated that alcohol can stimulate acid sphingomyelinase activity leading to a decrease of sphingomyelins.[39–41]

The variations observed for lipid metabolites were consistent with the observation that cell membranes are damaged because of smoking,[42] and because these lipids are major components of cell membranes, they are released into the circulation. Free radicals in cigarette smoke are thought to damage lipids,[43] particularly unsaturated fatty acids. They showed that current cigarette smokers had higher measures of lipid peroxidation than nonsmokers. Accordingly, the finding of increased lipid peroxidation in smokers supports the hypothesis that smoking increases free radical–mediated oxidative damage of lipids, an accepted risk factor for atherosclerotic CVD.[43] We showed that higher concentrations of diacyl PC and lower concentrations of acyl-alkyl PC (both associated with higher tobacco consumption) were associated with a lower level of RAGE. It was previously shown that a decreased level of RAGE is a biomarker for deficient inflammatory control in humans.

The diet score obtained in the food frequency questionnaire showed a positive association with a diacyl PC (PC aa C42:2) cross-sectionally. This lipid was not seen in association with any of the other risk factors. However, this is in accordance with a previous study that reported the association between a standard diet (in contrast with high fat diet) and higher levels of PC aa C42:2 in mice.[44] The long-term effects shown on lipid metabolism associated with healthy diet are also in accordance with the literature. A healthy diet, as determined by high consumption of fruit and vegetables, has been previously strongly associated in women with acyl-alkyl C40:6.[45]

Sex-Specific Effects

In this study, we found sex-specific effects of smoking and BMI on metabolite profiles, specifically on acylC metabolism. This result supports the assumption that differences in effects of BMI and smoking in men and women are not solely based on their sex-driven frequencies but are also biologically driven, that is, positive associations between acylC and BMI for women and between acylC and smoking for men.

Strengths and Limitations

We considered several modifiable CVD risk factors simultaneously and used a systematic targeted metabolomics approach with 134 metabolites in a large well-characterized population-based cohort. However, the approach mainly focuses on 3 classes of lipid metabolites and, thus, is not representative of the whole metabolome. This is an association study; results may reflect the underlying population composition and not a causal relationship between risk factor and metabolites, but prospective analyses suggest changes that are biologically plausible as causal factors. Nonetheless, the longitudinal design of our study makes these results more significant and provides additional support to the hypothesis that modifiable CVD risk factors are a cause of changes in metabolite concentrations during a 4-year time period. These results are further supported by the sensitivity analysis that reinforce that observed changes in metabolites are not because of changes in risk factors. The characterization of the risk factors was done by questionnaires and self-reports. There are several limitations associated with the use of questionnaires as recall bias, poor specificity, or missing values. An additional limitation is inherent to the CARLA cohort, which was designed to study cardiovascular risk factors in the general elderly population, and accordingly participants aged 45 to 83 years at baseline. This cohort is also characterized by a pre-eminent prevalence of risk factors, particularly hypertension.[46] An additional remark needs to be mentioned in that the observed effects on metabolite profiles for some baseline risk

factors (BMI and alcohol) were much stronger than those for physical activity. This could be because of the fact that BMI and alcohol have long-term consequences, whereas sport is more variable and therefore have also short-term and unstable consequences. However, it needs to be taken into consideration that there are important differences in the quality of data acquisition: BMI, tobacco use, and alcohol consumption were thoroughly acquired. A further limitation was the use of self-report to define type 2 diabetes mellitus, an exclusion criterion for this study because of the different metabolism in those participants, which might have led to undetected cases, and those undetected cases could bias the results of this study. In general, the specificity of self-reported type 2 diabetes mellitus can be considered as high, whereas sensitivity is relatively low.[47] Finally, the use of serum instead of plasma for the determination of metabolite concentrations could lead to bias. It has been shown that lysoPC levels are higher in serum than in plasma samples, suggesting that the clotting process influences serum lipid metabolite levels.[48] In a recent study comparing human serum and plasma using the Biocrates platform, it was found that higher concentrations were always measured in serum than in plasma; yet as long as the same blood preparation procedure is used, plasma or serum generate similar results in clinical and biological studies.[49] Furthermore, serum was recommended because it provides more sensitive results in biomarker detection.

Conclusions

The longitudinal analysis of the effects of classic CVD risks factors on human metabolism suggests clear sex-specific and general alterations of lipid metabolism associated with BMI, alcohol, and tobacco with lesser effects on acylC. The most obvious long-term metabolic consequences are for smoking, alcohol, diet, and BMI on diacyl PC, acyl-alkyl PC, and nonessential AA levels, respectively. These risk factors are modifiable and support intervention strategies to directly alter the course of pathological pathways leading to chronic disease.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Metabolomics research can identify distinct metabolic patterns in individuals affected by pathological conditions like cardiovascular disorders. This technology has been also used to study the effects of several classic modifiable cardiovascular risk factors on metabolism in different population-based cohorts. However, the effect of all these risk factors taken together, the long-term effects of these risk factors on the human metabolism, and whether or not there are sex-specific differences in these associations have not yet been investigated. We evaluated associations between metabolite concentrations and 5 modifiable risk factors (BMI, alcohol consumption, smoking, diet and exercise) in 1030 healthy adults from a population-based cohort. The longitudinal analysis of the effects of modifiable risks factors on human metabolism suggest clear sex-specific and general alterations of lipid metabolism associated with BMI, alcohol and tobacco with lesser effects on acylcarnitines. Sex-specific effects of smoking and BMI were found specifically related to acylcarnitine metabolism: in women higher BMI and in men more pack-years were associated with increases in acylcarnitines. The most obvious long-term metabolic consequences are for smoking, alcohol, diet and BMI on diacyl-, acyl-alkyl phosphatidylcholines and nonessential amino acid levels, respectively. These risk factors are modifiable and support intervention strategies to directly alter the course of pathological pathways leading to chronic disease.

Cardiovascular Risk Factors Associated With Blood Metabolite Concentrations and Their Alterations During a 4-Year Period in a Population-Based Cohort

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Supplemental material

Supplemental methods

Quality Control of the Metabolomics Dataset

First, 2 metabolites (lysoPC a C6:0 and PC ae C38:1) were excluded due to the number of missing values within lab analyses that exceeded 5% (values =0). The remaining missing values (1‰ of all values) were imputed using the SAS procedure MI with the MCMC (Markov chain Monte Carlo) method. Multiple imputation is a method to treat missing values in which each variable is estimated using a regression model conditional on all the other variables iteratively looping through all the variables with missing data [1]. Imputations were done with minimum and maximum values defined from the CARLA population and every single imputation was plausibility checked. Additionally, 27 (13 acylC, 9 PC and 5 SM) further metabolites were excluded from the analysis as their experimental variation assessed through the coefficient of variation (CV) of 173 measured aliquots of a reference plasma sample (5 on each plate) exceeded 25%. Reference plasma derived from a pool of plasma from 6 healthy British individuals in the age range 20-57 years including 3 men and 3 women. Since blood samples were analysed on thirty-five plates (batches), a so-called batch variable was included in analyses as a random factor in order to avoid possible effects due to technical issues or different time points of analyses. No outliers, defined as greater than mean ± 5 standard deviations of the particular metabolite over the whole population, were found [2].

Statistical analysis: conservation index

A conservation index was calculated for metabotypes (the set of metabolite concentrations for an individual), defined as the relative rank of the longitudinal metabotype intra-correlation of that individual with respect to all longitudinal metabotype inter-correlations of that individual with all other individuals from the same study cohort, following [3]. To calculate this index, the intra-correlations are converted to ranks to measure a metabotype's or metabolite's similarity to itself when compared to its similarity to other metabotypes or metabolites. It is

calculated as $1 - ((rank(i) - 1)/(N - 1))$, where N is number of metabotypes and (*i)* is each participant. This index quantifies the comparison of intra-correlations to inter-correlations, yielding a value in the range [0,1].

Supplemental results

Metabotype conservation over time

The longitudinal design of our study allows the investigation of whether changes in risk factors associate with overall changes in metabotype. To this end, we used the metabotype conservation index as a measure of human metabotype persistence over 4 years. The conservation index ranks an individual metabotype's longitudinal intra-correlation considering the longitudinal correlation with all other individuals' metabotypes. A fully conserved metabotype (a conservation index of 1) was observed for 985 of the 1030 study participants, which indicates that 96% of the study participants could be uniquely identified after 4 years based on information about their metabolic profiles alone. Conversely, only 4 participants (0.4%) of the metabotypes had a conservation index below 0.7; i.e., they considerably changed their metabolic profiles over the 4-year period (Supplementary Figure 2). For those 4 participants with a conservation index below 0.7 no changes in risk factors could be seen, and no association could be found between changes in metabotype and changes in risk factors.

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Supplemental Table 1. List of metabolites (N=134) with raw values (before transformation) and sex-stratified correlation between baseline and follow-up values per metabolite.

			Men, n=534					Women, n=496				
Biochemical name	**Short name**	**Metabolite class**	**Baseline**		**Follow-up**		**r**	**Baseline**		**Follow-up**		**r**
			Mean	**Std Dev**	**Mean**	**Std Dev**		**Mean**	**Std Dev**	**Mean**	**Std Dev**	
Carnitine	C0	Acylcarnitines	48.18	13.34	49.11	13.27	0.67	42.46	10.26	45.37	11.11	0.54
Acetylcarnitine	C2	Acylcarnitines	8.10	3.92	8.01	3.31	0.52	8.22	3.70	8.81	3.58	0.42
Propionylcarnitine	C3	Acylcarnitines	0.48	0.18	0.49	0.23	0.55	0.40	0.20	0.41	0.18	0.40
Butyrylcarnitine	C4	Acylcarnitines	0.25	0.10	0.25	0.11	0.68	0.22	0.09	0.24	0.11	0.70
Valerylcarnitine	C5	Acylcarnitines	0.18	0.06	0.18	0.07	0.41	0.15	0.05	0.16	0.08	0.40
Tiglylcarnitine	C5:1	Acylcarnitines	0.04	0.01	0.04	0.01	0.44	0.04	0.01	0.04	0.01	0.49
Octanoylcarnitine	C8	Acylcarnitines	0.15	0.07	0.16	0.08	0.54	0.15	0.06	0.15	0.06	0.69
Octenoylcarnitine	C8:1	Acylcarnitines	0.15	0.08	0.15	0.08	0.50	0.14	0.07	0.14	0.08	0.45
Nonaylcarnitine	C9	Acylcarnitines	0.04	0.03	0.05	0.03	0.60	0.04	0.02	0.04	0.02	0.51
Decanoylcarnitine	C10	Acylcarnitines	0.25	0.11	0.25	0.12	0.52	0.24	0.11	0.25	0.10	0.62
Decenoylcarnitine	C10:1	Acylcarnitines	0.14	0.05	0.13	0.05	0.52	0.13	0.05	0.13	0.04	0.58
Decadienylcarnitine	C10:2	Acylcarnitines	0.05	0.02	0.05	0.02	0.46	0.05	0.01	0.05	0.02	0.33
Dodecanoylcarnitine	C12	Acylcarnitines	0.12	0.05	0.11	0.05	0.57	0.10	0.04	0.11	0.04	0.53

Dodecenoylcarnitine	C12:1	Acylcarnitines	0.11	0.05	0.11	0.04	0.64	0.10	0.04	0.11	0.04	0.63
Tetradecadienylcarnitine	C14:2	Acylcarnitines	0.02	0.01	0.02	0.01	0.49	0.02	0.01	0.02	0.01	0.39
Hexadecanoylcarnitine	C16	Acylcarnitines	0.14	0.04	0.14	0.04	0.58	0.12	0.04	0.13	0.03	0.49
Hexadecadienoylcarnitine	C16:2	Acylcarnitines	0.01	0.00	0.01	0.00	0.39	0.01	0.00	0.01	0.00	0.24
Octadecanoylcarnitine	C18	Acylcarnitines	0.06	0.02	0.06	0.02	0.58	0.05	0.02	0.06	0.02	0.51
Octadecenoylcarnitine	C18:1	Acylcarnitines	0.14	0.05	0.16	0.05	0.54	0.13	0.05	0.15	0.05	0.47
Octadecadienylcarnitine	C18:2	Acylcarnitines	0.04	0.02	0.05	0.02	0.54	0.04	0.01	0.05	0.02	0.41
Hydroxybutyrylcarnitine	C4.OH (and C3-DC)	hydroxy- and dicarboxy-acylcarnitines	0.08	0.06	0.08	0.06	0.43	0.06	0.03	0.07	0.02	0.45
Hydroxyvalerylcarnitine	C5.OH (and C3.DC.M)	hydroxy- and dicarboxy-acylcarnitines	0.04	0.01	0.04	0.01	0.56	0.04	0.01	0.04	0.01	0.48
Hexanoylcarnitine	C6 (and C4.1.DC)	hydroxy- and dicarboxy-acylcarnitines	0.09	0.03	0.09	0.03	0.55	0.08	0.03	0.09	0.03	0.58
Pimeloylcarnitine	C7-DC	hydroxy- and dicarboxy-acylcarnitines	0.04	0.02	0.04	0.02	0.47	0.03	0.01	0.03	0.01	0.54
Hydroxytetradecenoylcarnitine	C14:1-OH	hydroxy- and dicarboxy-acylcarnitines	0.01	0.00	0.01	0.00	0.43	0.01	0.00	0.01	0.01	0.19
Hydroxyhexadecenoylcarnitine	C16:1-OH	hydroxy- and dicarboxy-acylcarnitines	0.01	0.00	0.01	0.00	0.46	0.01	0.00	0.01	0.01	0.24
Hydroxyhexadecadienoylcarnitine	C16:2-OH	hydroxy- and dicarboxy-	0.01	0.00	0.01	0.00	0.39	0.01	0.00	0.01	0.00	0.27

		acylcarnitines										
Hydroxyoctadecenoylcarnitine	C18:1-OH	hydroxy- and dicarboxy-acylcarnitines	0.01	0.00	0.01	0.00	0.36	0.01	0.00	0.01	0.01	0.30
Arginine	Arg	Amino acids	123.97	21.98	116.89	22.86	0.54	122.77	22.78	117.73	21.52	0.48
Glutamine	Gln	Amino acids	600.80	101.82	606.20	105.77	0.58	580.64	108.26	599.79	107.43	0.53
Glycine	Gly	Amino acids	243.63	55.64	251.56	59.35	0.67	289.44	102.70	297.62	95.99	0.78
Histidine	His	Amino acids	83.85	15.58	85.04	15.74	0.52	81.28	16.31	82.78	16.09	0.53
Methionine	Met	Amino acids	32.69	8.10	32.96	7.94	0.43	31.12	12.77	31.09	7.45	0.31
Ornithine	Orn	Amino acids	69.92	18.74	89.93	22.73	0.49	67.43	18.47	88.69	21.89	0.41
Phenylalanine	Phe	Amino acids	54.71	10.87	57.61	12.01	0.49	54.06	10.80	57.09	11.01	0.44
Proline	Pro	Amino acids	244.32	76.70	250.65	76.01	0.66	215.90	70.39	227.57	74.45	0.55
Serine	Ser	Amino acids	108.52	24.90	114.76	26.08	0.63	116.75	27.70	123.50	27.95	0.64
Threonine	Thr	Amino acids	112.11	30.44	113.62	30.49	0.62	109.80	30.73	112.78	29.22	0.50
Tryptophan	Trp	Amino acids	89.05	13.81	88.92	14.11	0.56	85.11	12.85	86.55	13.79	0.45
Tyrosine	Tyr	Amino acids	100.99	27.02	103.50	27.67	0.55	100.69	30.23	105.85	32.61	0.49
Valine	Val	Amino acids	201.25	65.86	205.74	68.69	0.75	182.27	65.37	190.37	65.69	0.75
Leucine and isoleucine	xLeu	Amino acids	239.37	68.13	241.10	63.50	0.35	212.07	61.82	219.49	64.74	0.37
Lysophosphatidylcholine (acyl) C14:0	lysoPC a C14:0	Glycerophospholipids	4.17	0.85	4.21	0.89	0.72	4.19	0.83	4.32	0.82	0.61

Lysophosphatidylcholine (acyl) C16:0	lysoPC a C16:0	Glycerophospholipids	92.48	25.53	95.66	28.25	0.68	84.54	23.91	91.80	22.97	0.65
Lysophosphatidylcholine (acyl) C16:1	lysoPC a C16:1	Glycerophospholipids	3.06	1.35	3.06	1.49	0.63	3.02	1.11	3.18	1.06	0.65
Lysophosphatidylcholine (acyl) C17:0	lysoPC a C17:0	Glycerophospholipids	1.47	0.54	1.53	0.58	0.73	1.57	0.58	1.70	0.57	0.67
Lysophosphatidylcholine (acyl) C18:0	lysoPC a C18:0	Glycerophospholipids	25.76	7.29	26.82	8.14	0.66	24.33	7.27	27.21	7.12	0.53
Lysophosphatidylcholine (acyl) C18:1	lysoPC a C18:1	Glycerophospholipids	21.36	7.73	20.68	7.78	0.60	19.27	6.25	19.69	5.73	0.55
Lysophosphatidylcholine (acyl) C18:2	lysoPC a C18:2	Glycerophospholipids	37.59	14.56	34.93	13.88	0.52	33.58	13.05	32.86	11.96	0.48
LysoPhosphatidylcholine (acyl) C20:3	lysoPC a C20:3	Glycerophospholipids	2.65	1.03	2.51	1.00	0.56	2.48	0.87	2.52	0.84	0.43
Lysophosphatidylcholine (acyl) C20:4	lysoPC a C20:4	Glycerophospholipids	6.73	2.42	6.62	2.46	0.61	5.94	2.00	6.24	1.93	0.54
Phosphatidylcholine (diacyl) C26:0	PC aa C26:0	Glycerophospholipids	0.76	0.23	0.74	0.21	0.75	0.78	0.24	0.77	0.22	0.80
Phosphatidylcholine (diacyl) C28:1	PC aa C28:1	Glycerophospholipids	3.39	0.92	3.39	0.96	0.72	4.07	1.11	4.22	1.08	0.65
Phosphatidylcholine (diacyl) C30:0	PC aa C30:0	Glycerophospholipids	6.01	2.15	5.85	2.10	0.56	6.68	2.27	6.77	2.18	0.46
Phosphatidylcholine (diacyl) C32:0	PC aa C32:0	Glycerophospholipids	15.19	3.88	15.00	3.94	0.63	15.42	4.08	15.75	3.93	0.66
Phosphatidylcholine (diacyl) C32:1	PC aa C32:1	Glycerophospholipids	20.51	12.90	19.23	12.15	0.64	21.40	12.32	21.40	11.06	0.69

Phosphatidylcholine (diacyl) C32:2	PC aa C32:2	Glycerophospholipids	5.14	1.78	4.92	1.84	0.59	6.10	2.25	6.07	2.10	0.54
Phosphatidylcholine (diacyl) C32:3	PC aa C32:3	Glycerophospholipids	0.57	0.18	0.53	0.18	0.69	0.71	0.24	0.68	0.23	0.72
Phosphatidylcholine (diacyl) C34:1	PC aa C34:1	Glycerophospholipids	247.33	75.52	241.41	74.94	0.64	247.35	75.73	251.31	73.40	0.72
Phosphatidylcholine (diacyl) C34:2	PC aa C34:2	Glycerophospholipids	408.16	113.47	397.06	116.90	0.78	414.45	123.03	416.37	121.89	0.78
Phosphatidylcholine (diacyl) C34:3	PC aa C34:3	Glycerophospholipids	19.72	6.69	18.34	6.55	0.52	21.90	6.82	21.55	6.65	0.60
Phosphatidylcholine (diacyl) C34:4	PC aa C34:4	Glycerophospholipids	2.50	0.96	2.36	0.93	0.59	2.84	1.09	2.84	1.06	0.61
Phosphatidylcholine (diacyl) C36:0	PC aa C36:0	Glycerophospholipids	3.21	1.02	3.17	0.97	0.61	3.47	1.10	3.51	1.09	0.67
Phosphatidylcholine (diacyl) C36:1	PC aa C36:1	Glycerophospholipids	60.88	18.73	58.44	19.05	0.59	62.88	16.97	64.10	17.36	0.49
Phosphatidylcholine (diacyl) C36:2	PC aa C36:2	Glycerophospholipids	262.38	67.51	253.87	70.43	0.71	272.95	76.52	276.98	76.73	0.71
Phosphatidylcholine (diacyl) C36:3	PC aa C36:3	Glycerophospholipids	159.54	41.56	153.63	43.06	0.66	169.55	46.25	169.00	44.44	0.68
Phosphatidylcholine (diacyl) C36:4	PC aa C36:4	Glycerophospholipids	218.68	63.06	213.49	62.85	0.75	223.51	63.40	226.76	61.60	0.74
Phosphatidylcholine (diacyl) C36:5	PC aa C36:5	Glycerophospholipids	39.38	21.16	37.97	20.85	0.44	39.37	20.44	39.64	19.14	0.53
Phosphatidylcholine (diacyl) C36:6	PC aa C36:6	Glycerophospholipids	1.43	0.62	1.36	0.62	0.48	1.68	0.74	1.64	0.69	0.61
Phosphatidylcholine (diacyl) C38:0	PC aa C38:0	Glycerophospholipids	3.18	0.99	3.12	0.99	0.65	3.48	1.18	3.49	1.19	0.70
Phosphatidylcholine (diacyl) C38:3	PC aa C38:3	Glycerophospholipids	60.13	17.06	58.83	17.75	0.67	65.93	17.39	68.00	17.19	0.62
Phosphatidylcholine (diacyl) C38:4	PC aa C38:4	Glycerophospholipids	122.85	36.88	120.07	37.10	0.74	129.00	35.28	133.23	34.48	0.69
Phosphatidylcholine (diacyl) C38:5	PC aa C38:5	Glycerophospholipids	64.66	20.24	62.36	19.76	0.63	67.09	19.08	68.14	18.44	0.62
Phosphatidylcholine (diacyl) C38:6	PC aa C38:6	Glycerophospholipids	103.17	33.54	101.32	33.29	0.62	110.00	35.72	110.23	35.93	0.65
Phosphatidylcholine (diacyl) C40:1	PC aa C40:1	Glycerophospholipids	0.40	0.10	0.39	0.10	0.45	0.41	0.10	0.41	0.11	0.51

Phosphatidylcholine (diacyl) C40:2	PC aa C40:2	Glycerophospholipids	0.39	0.13	0.38	0.13	0.36	0.38	0.12	0.39	0.13	0.31
Phosphatidylcholine (diacyl) C40:3	PC aa C40:3	Glycerophospholipids	0.68	0.21	0.67	0.21	0.45	0.69	0.22	0.71	0.22	0.39
Phosphatidylcholine (diacyl) C40:4	PC aa C40:4	Glycerophospholipids	4.18	1.54	4.06	1.52	0.66	4.18	1.24	4.30	1.16	0.58
Phosphatidylcholine (diacyl) C40:5	PC aa C40:5	Glycerophospholipids	14.15	5.06	13.80	5.04	0.67	14.13	4.23	14.75	4.22	0.59
Phosphatidylcholine (diacyl) C40:6	PC aa C40:6	Glycerophospholipids	36.86	13.53	36.12	13.43	0.64	39.25	13.56	40.21	14.38	0.65
Phosphatidylcholine (diacyl) C42:0	PC aa C42:0	Glycerophospholipids	0.57	0.18	0.57	0.19	0.70	0.64	0.22	0.65	0.22	0.70
Phosphatidylcholine (diacyl) C42:1	PC aa C42:1	Glycerophospholipids	0.28	0.08	0.28	0.08	0.67	0.31	0.10	0.31	0.09	0.69
Phosphatidylcholine (diacyl) C42:2	PC aa C42:2	Glycerophospholipids	0.28	0.09	0.27	0.08	0.48	0.28	0.09	0.28	0.08	0.47
Phosphatidylcholine (diacyl) C42:4	PC aa C42:4	Glycerophospholipids	0.19	0.05	0.19	0.05	0.59	0.19	0.05	0.20	0.05	0.48
Phosphatidylcholine (diacyl) C42:5	PC aa C42:5	Glycerophospholipids	0.48	0.18	0.48	0.18	0.48	0.49	0.16	0.50	0.17	0.35
Phosphatidylcholine (diacyl) C42:6	PC aa C42:6	Glycerophospholipids	0.61	0.20	0.58	0.19	0.56	0.65	0.19	0.65	0.18	0.50
Phosphatidylcholine (acyl-alkyl)C30:0	PC ae C30:0	Glycerophospholipids	0.40	0.13	0.39	0.13	0.60	0.46	0.16	0.46	0.15	0.56
Phosphatidylcholine (acyl-alkyl) C30:2	PC ae C30:2	Glycerophospholipids	0.17	0.06	0.17	0.06	0.80	0.21	0.07	0.22	0.08	0.77
Phosphatidylcholine (acyl-alkyl) C32:1	PC ae C32:1	Glycerophospholipids	2.78	0.68	2.75	0.72	0.63	3.02	0.78	3.08	0.76	0.68
Phosphatidylcholine (acyl-alkyl) C32:2	PC ae C32:2	Glycerophospholipids	0.67	0.18	0.67	0.19	0.69	0.82	0.23	0.82	0.23	0.73
Phosphatidylcholine (acyl-alkyl)	PC ae C34:0	Glycerophospholipids	1.70	0.54	1.65	0.54	0.60	1.91	0.61	1.96	0.62	0.63

C34:0												
Phosphatidylcholine (acyl-alkyl) C34:1	PC ae C34:1	Glycerophospholipids	10.13	2.52	9.97	2.64	0.61	11.55	3.07	11.78	3.14	0.65
Phosphatidylcholine (acyl-alkyl) C34:2	PC ae C34:2	Glycerophospholipids	12.02	3.56	11.68	3.49	0.60	13.53	4.08	13.57	3.92	0.61
Phosphatidylcholine (acyl-alkyl) C34:3	PC ae C34:3	Glycerophospholipids	7.94	2.43	7.81	2.46	0.68	8.98	2.90	9.09	2.84	0.70
Phosphatidylcholine (acyl-alkyl) C36:0	PC ae C36:0	Glycerophospholipids	1.01	0.31	0.98	0.31	0.65	1.03	0.32	1.04	0.34	0.66
Phosphatidylcholine (acyl-alkyl) C36:1	PC ae C36:1	Glycerophospholipids	8.13	2.18	7.95	2.26	0.65	9.47	2.58	9.67	2.69	0.64
Phosphatidylcholine (acyl-alkyl) C36:2	PC ae C36:2	Glycerophospholipids	15.33	4.24	14.89	4.38	0.71	18.13	5.04	18.26	5.13	0.68
Phosphatidylcholine (acyl-alkyl) C36:3	PC ae C36:3	Glycerophospholipids	9.07	2.57	8.78	2.49	0.61	10.06	2.90	10.01	2.81	0.64
Phosphatidylcholine (acyl-alkyl) C36:4	PC ae C36:4	Glycerophospholipids	19.79	6.32	19.22	5.72	0.64	19.72	5.94	20.12	5.77	0.63
Phosphatidylcholine (acyl-alkyl) C36:5	PC ae C36:5	Glycerophospholipids	13.88	4.16	13.70	4.05	0.68	14.05	4.33	14.53	4.18	0.70
Phosphatidylcholine (acyl-alkyl) C38:0	PC ae C38:0	Glycerophospholipids	2.29	0.81	2.17	0.81	0.54	2.57	0.93	2.52	0.89	0.66

Phosphatidylcholine (acyl-alkyl) C38:2	PC ae C38:2	Glycerophospholipids	2.29	0.56	2.22	0.57	0.57	2.57	0.66	2.60	0.70	0.57
Phosphatidylcholine (acyl-alkyl) C38:3	PC ae C38:3	Glycerophospholipids	4.38	1.09	4.25	1.09	0.63	5.20	1.31	5.26	1.32	0.61
Phosphatidylcholine (acyl-alkyl) C38:4	PC ae C38:4	Glycerophospholipids	13.68	3.41	13.37	3.37	0.66	14.68	3.77	14.97	3.66	0.65
Phosphatidylcholine (acyl-alkyl) C38:5	PC ae C38:5	Glycerophospholipids	20.27	5.12	19.85	5.06	0.65	20.52	5.25	20.90	5.12	0.65
Phosphatidylcholine (acyl-alkyl) C38:6	PC ae C38:6	Glycerophospholipids	8.46	2.42	8.23	2.34	0.56	9.12	2.67	9.21	2.61	0.63
Phosphatidylcholine (acyl-alkyl) C40:0	PC ae C40:0	Glycerophospholipids	6.00	1.68	5.71	1.71	0.66	6.62	1.98	6.45	1.91	0.68
Phosphatidylcholine (acyl-alkyl) C40:1	PC ae C40:1	Glycerophospholipids	1.47	0.41	1.40	0.40	0.57	1.49	0.44	1.49	0.44	0.63
Phosphatidylcholine (acyl-alkyl) C40:2	PC ae C40:2	Glycerophospholipids	2.10	0.59	2.04	0.58	0.65	2.38	0.64	2.42	0.67	0.66
Phosphatidylcholine (acyl-alkyl) C40:3	PC ae C40:3	Glycerophospholipids	1.04	0.23	1.02	0.23	0.62	1.22	0.27	1.23	0.28	0.60
Phosphatidylcholine (acyl-alkyl) C40:4	PC ae C40:4	Glycerophospholipids	2.54	0.59	2.48	0.59	0.65	2.70	0.65	2.75	0.64	0.63
Phosphatidylcholine (acyl-alkyl)	PC ae C40:5	Glycerophospholipids	4.06	0.90	3.91	0.90	0.60	4.31	1.07	4.30	1.00	0.63

C40:5												
Phosphatidylcholine (acyl-alkyl) C40:6	PC ae C40:6	Glycerophospholipids	5.28	1.44	5.16	1.42	0.63	5.98	1.83	5.99	1.81	0.68
Phosphatidylcholine (acyl-alkyl) C42:0	PC ae C42:0	Glycerophospholipids	0.49	0.10	0.48	0.10	0.59	0.49	0.10	0.50	0.11	0.51
Phosphatidylcholine (acyl-alkyl) C42:1	PC ae C42:1	Glycerophospholipids	0.44	0.13	0.42	0.13	0.61	0.45	0.11	0.46	0.11	0.62
Phosphatidylcholine (acyl-alkyl) C42:2	PC ae C42:2	Glycerophospholipids	0.67	0.17	0.65	0.18	0.50	0.72	0.20	0.73	0.20	0.54
Phosphatidylcholine (acyl-alkyl) C42:3	PC ae C42:3	Glycerophospholipids	0.77	0.19	0.76	0.20	0.55	0.84	0.23	0.84	0.23	0.62
Phosphatidylcholine (acyl-alkyl) C42:4	PC ae C42:4	Glycerophospholipids	0.97	0.26	0.95	0.27	0.68	1.02	0.28	1.04	0.27	0.61
Phosphatidylcholine (acyl-alkyl) C42:5	PC ae C42:5	Glycerophospholipids	2.24	0.54	2.18	0.54	0.72	2.39	0.60	2.38	0.58	0.67
Phosphatidylcholine (acyl-alkyl) C44:3	PC ae C44:3	Glycerophospholipids	0.13	0.04	0.13	0.04	0.55	0.14	0.04	0.14	0.04	0.58
Phosphatidylcholine (acyl-alkyl) C44:4	PC ae C44:4	Glycerophospholipids	0.42	0.12	0.41	0.12	0.73	0.44	0.13	0.46	0.13	0.62
Phosphatidylcholine (acyl-alkyl) C44:5	PC ae C44:5	Glycerophospholipids	1.93	0.60	1.94	0.62	0.76	2.01	0.62	2.06	0.61	0.71

Phosphatidylcholine (acyl-alkyl) C44:6	PC ae C44:6	Glycerophospholipids	1.26	0.39	1.27	0.40	0.75	1.34	0.42	1.37	0.41	0.71
Hydroxysphingomyeline C14:1	SM (OH) C14:1	Sphingolipids	7.47	2.68	7.52	2.73	0.83	9.24	3.05	9.68	3.11	0.78
Hydroxysphingomyeline C16:1	SM (OH) C16:1	Sphingolipids	4.04	1.42	4.06	1.47	0.81	5.03	1.64	5.23	1.68	0.76
Hydroxysphingomyeline C22:1	SM (OH) C22:1	Sphingolipids	16.60	5.90	16.51	6.00	0.80	19.65	6.35	20.27	6.48	0.76
Hydroxysphingomyeline C22:2	SM (OH) C22:2	Sphingolipids	13.42	4.77	13.44	4.81	0.81	17.51	5.70	18.14	5.80	0.78
Sphingomyeline C16:0	SM C16:0	Sphingolipids	126.60	34.58	127.17	36.07	0.79	136.31	35.04	141.07	34.98	0.74
Sphingomyeline C16:1	SM C16:1	Sphingolipids	19.57	5.47	19.54	5.53	0.79	23.57	6.32	24.41	6.16	0.74
Sphingomyeline C18:0	SM C18:0	Sphingolipids	31.75	9.17	31.73	9.48	0.75	36.35	10.07	37.65	10.02	0.73
Sphingomyeline C18:1	SM C18:1	Sphingolipids	14.22	4.41	13.99	4.37	0.78	18.07	5.38	18.45	5.24	0.75
Sphingomyeline C24:0	SM C24:0	Sphingolipids	29.55	9.86	28.89	9.86	0.79	30.18	9.02	30.69	8.99	0.74
Sphingomyeline C24:1	SM C24:1	Sphingolipids	72.82	21.90	72.31	23.26	0.75	75.45	20.88	77.01	21.21	0.73
Hexose	H1	Sugars	6293.00	1210.00	6102.00	1474.00	0.54	5974.00	1073.00	5852.00	1388.00	0.40

Supplemental table 2. Cross-sectional associations between lifestyle risk factors and individual metabolites.

Metabolite	BMI		Diet		Alcohol		Pack-years		Sport	
	beta (SE)	p-value (FDR)	beta (SE)	p-value (FDR)	beta (SE)	p-value (FDR)	beta (SE)	p-value (FDR)	beta (SE)	p-value (FDR)
C0	**0.03 (0.01)**	**0.0004 (0.01)**	-0.01 (0.02)	0.72 (0.92)	0.11 (0.05)	0.03 (0.23)	0.07 (0.05)	0.13 (0.48)	-0.42 (0.32)	0.19 (0.56)
C2	**0.02 (0.01)**	**0.002 (0.04)**	0.02 (0.02)	0.47 (0.80)	0.03 (0.05)	0.53 (0.83)	**0.10 (0.02)**	**0.0005 (0.02)**	-0.50 (0.33)	0.14 (0.49)
C4-OH (and C3-DC)	**0.02 (0.01)**	**<.0001 (0.001)**	0.03 (0.02)	0.16 (0.52)	0.09 (0.05)	0.08 (0.37)	**0.09 (0.02)**	**0.001 (0.03)**	-0.27 (0.32)	0.41 (0.76)
C4	-0.03 (0.02)	0.14 (0.49)	-0.03 (0.02)	0.21 (0.60)	0.01 (0.05)	0.92 (0.98)	**0.06 (0.02)**	**0.002 (<0.05)**	-0.39 (0.34)	0.25 (0.64)
C6 (and C4:1-DC)	-0.01 (0.02)	0.42 (0.77)	0.01 (0.02)	0.68 (0.90)	0.12 (0.05)	0.02 (0.18)	**0.12 (0.02)**	**0.001 (0.03)**	-0.32 (0.33)	0.33 (0.70)
C7-DC	-0.03 (0.02)	0.05 (0.30)	-0.01 (0.02)	0.53 (0.83)	0.08 (0.05)	0.12 (0.47)	**0.16 (0.05)**	**0.0008 (0.02)**	-0.20 (0.32)	0.53 (0.84)
C8	-0.01 (0.02)	0.78 (0.94)	-0.02 (0.02)	0.28 (0.66)	0.05 (0.05)	0.35 (0.72)	**0.17 (0.05)**	**0.0007 (0.02)**	-0.54 (0.34)	0.11 (0.44)
C10	-0.02 (0.02)	0.37 (0.74)	-0.02 (0.02)	0.32 (0.70)	0.03 (0.05)	0.52 (0.83)	**0.17 (0.05)**	**0.0003 (0.01)**	-0.42 (0.33)	0.20 (0.58)
C16:2	-0.03 (0.02)	0.09 (41)	0.03 (0.02)	0.10 (0.42)	**0.07 (0.02)**	**0.0003 (0.01)**	0.09 (0.05)	0.08 (0.38)	-0.52 (0.35)	0.14 (0.49)
C16:2-OH	-0.02 (0.02)	0.24 (0.62)	-0.01 (0.02)	0.57 (0.85)	0.11 (0.05)	0.03 (0.24)	-0.07 (0.03)	0.002 (0.05)	0.20 (0.34)	0.54 (0.84)
Arg	-0.05 (0.02)	0.02 (0.17)	0.02 (0.02)	0.31 (0.68)	-0.12 (0.05)	0.03 (0.23)	**0.16 (0.05)**	**0.001 (0.03)**	**-0.55 (0.15)**	**<.0001 (0.004)**
Gln	**-0.06 (0.02)**	**0.001 (0.03)**	0.02 (0.02)	0.36 (0.73)	-0.09 (0.05)	0.10 (0.42)	0.02 (0.05)	0.71 (0.91)	-0.41 (0.33)	0.22 (0.60)
Gly	**-0.08 (0.02)**	**<.0001 (0.0004)**	0.01 (0.02)	0.66 (0.89)	-0.08 (0.05)	0.12 (0.45)	-0.01 (0.05)	0.92 (0.98)	-0.35 (0.34)	0.30 (0.68)
Ser	**-0.04 (0.01)**	**0.0002 (0.01)**	0.04 (0.02)	0.08 (0.39)	-0.15 (0.05)	0.003 (0.06)	0.08 (0.05)	0.10 (0.41)	-0.30 (0.32)	0.35 (0.72)
Tyr	**0.09 (0.02)**	**<.0001 (0.00004)**	0.06 (0.02)	0.01 (0.11)	-0.03 (0.05)	0.52 (0.83)	0.04 (0.05)	0.43 (0.78)	0.12 (0.33)	0.72 (0.91)
lysoPC a C16:1	-0.03 (0.02)	0.13 (0.47)	-0.01 (0.02)	0.51 (0.83)	**0.19 (0.05)**	**0.0001 (0.01)**	0.06 (0.04)	0.19 (0.57)	-0.20 (0.31)	0.53 (0.83)

lysoPC a C17:0	**-0.07 (0.02)**	**<.0001 (0.003)**	-0.02 (0.02)	0.45 (0.79)	**-0.27 (0.05)**	**<.0001 (<.0001)**	-0.11 (0.05)	0.01 (0.16)	0.34 (0.32)	0.29 (0.67)
lysoPC a C18:0	**-0.06 (0.02)**	**0.001 (0.03)**	-0.03 (0.02)	0.18 (0.56)	-0.04 (0.05)	0.40 (0.76)	-0.13 (0.05)	0.004 (0.07)	0.19 (0.32)	0.55 (0.85)
lysoPC a C18:1	**-0.05 (0.01)**	**<.0001 (0.004)**	-0.02 (0.02)	0.28 (0.66)	0.05 (0.05)	0.26 (0.65)	-0.07 (0.04)	0.09 (0.41)	-0.18 (0.31)	0.56 (0.85)
lysoPC a C18:2	**-0.06 (0.02)**	**0.0005 (0.01)**	-0.01 (0.02)	0.51 (0.83)	-0.05 (0.05)	0.29 (0.67)	**-0.16 (0.05)**	**0.0009 (0.02)**	0.23 (0.33)	0.49 (0.82)
PC aa C28:1	-0.01 (0.02)	0.47 (0.80)	0.02 (0.02)	0.25 (0.64)	-0.09 (0.05)	0.05 (0.30)	**-0.10 (0.02)**	**0.0006 (0.02)**	-0.66 (0.30)	0.03 (0.21)
PC aa C30:0	0.0002 (0.02)	0.99 (0.99)	0.01 (0.02)	0.70 (0.91)	-0.05 (0.05)	0.30 (0.68)	**0.05 (0.02)**	**0.0005 (0.02)**	-0.58 (0.31)	0.06 (0.34)
PC aa C32:0	-0.02 (0.02)	0.27 (0.66)	0.01 (0.02)	0.52 (0.83)	0.001 (0.05)	0.98 (0.99)	**0.07 (0.02)**	**0.002 (0.04)**	-0.63 (0.30)	0.04 (0.26)
PC aa C32:1	0.01 (0.02)	0.60 (0.86)	0.002 (0.02)	0.92 (0.98)	**0.23 (0.05)**	**<.0001 (<.0001)**	**0.11 (0.02)**	**0.0009 (0.02)**	-0.34 (0.29)	0.24 (0.62)
PC aa C34:1	-0.04 (0.01)	0.008 (0.11)	0.01 (0.02)	0.75 (0.93)	**0.16 (0.04)**	**0.0002 (0.007)**	**0.10 (0.02)**	**0.003 (<0.05)**	-0.40 (0.27)	0.14 (0.49)
PC aa C34:4	**0.05 (0.02)**	**0.002 (0.04)**	-0.001 (0.02)	0.96 (0.99)	0.09 (0.05)	0.07 (0.35)	0.06 (0.04)	0.18 (0.56)	-0.18 (0.30)	0.53 (0.84)
PC aa C36:1	-0.04 (0.01)	0.01 (0.12)	-0.001 (0.02)	0.95 (0.99)	0.08 (0.04)	0.05 (0.31)	**0.16 (0.04)**	**<.0001 (0.004)**	-0.80 (0.28)	0.004 (0.07)
PC aa C36:3	-0.004 (0.01)	0.81 (0.95)	-0.002 (0.02)	0.91 (0.98)	0.06 (0.04)	0.14 (0.49)	**0.13 (0.04)**	**0.001 (0.03)**	-0.38 (0.27)	0.16 (0.52)
PC aa C38:3	0.05 (0.02)	0.003 (0.05)	-0.02 (0.02)	0.21 (0.58)	0.05 (0.04)	0.28 (0.66)	**0.13 (0.04)**	**0.0008 (0.02)**	-0.66 (0.28)	0.02 (0.18)
PC aa C38:4	**0.06 (0.02)**	**0.0006 (0.02)**	-0.03 (0.02)	0.16 (0.52)	0.09 (0.05)	0.06 (0.34)	0.06 (0.04)	0.16 (0.52)	-0.47 (0.30)	0.12 (0.46)
PC aa C40:2	-0.06 (0.02)	0.002 (0.05)	0.03 (0.02)	0.12 (0.47)	-0.04 (0.05)	0.47 (0.80)	0.08 (0.05)	0.10 (0.42)	-0.19 (0.34)	0.56 (0.85)
PC aa C42:2	-0.04 (0.02)	0.02 (0.18)	**0.06 (0.02)**	**0.002 (0.04)**	-0.01 (0.05)	0.80 (0.95)	0.05 (0.05)	0.34 (0.71)	-0.66 (0.32)	0.04 (0.26)
PC ae C30:0	-0.03 (0.02)	0.06 (0.34)	-0.004 (0.02)	0.83 (0.96)	**-0.13 (0.02)**	**0.001 (0.03)**	**-0.13 (0.03)**	**0.0005 (0.01)**	-0.33 (0.01)	0.31 (0.69)
PC ae C30:2	-0.03 (0.01)	0.05 (0.30)	0.03 (0.02)	0.11 (0.43)	**-0.18 (0.04)**	**<.0001 (0.001)**	0.06 (0.04)	0.10 (0.43)	-0.35 (0.26)	0.18 (0.55)
PC ae C32:2	0.03 (0.01)	0.002 (0.05)	0.02 (0.02)	0.15 (0.50)	-0.05 (0.04)	0.21 (0.59)	**-0.04 (0.02)**	**0.0008 (0.02)**	-0.73 (0.27)	0.01 (0.10)

PC ae C34:0	-0.03 (0.02)	0.08 (0.38)	0.01 (0.02)	0.80 (0.94)	**-0.18 (0.05)**	**0.0002 (0.01)**	**-0.10 (0.02)**	**0.0004 (0.01)**	-0.59 (0.31)	0.06 (0.31)
PC ae C34:1	-0.05 (0.02)	0.004 (0.06)	-0.01 (0.02)	0.26 (0.64)	-0.13 (0.05)	0.004 (0.07)	**-0.06 (0.02)**	**0.0007 (0.02)**	-0.33 (0.29)	0.26 (0.64)
PC ae C34:2	-0.03 (0.02)	0.04 (0.26)	0.01 (0.02)	0.55 (0.84)	**-0.23 (0.05)**	**<.0001 (<.001)**	0.01 (0.04)	0.80 (0.94)	-0.23 (0.31)	0.46 (0.80)
PC ae C36:0	**-0.05 (0.02)**	**0.001 (0.03)**	0.11 (0.04)	0.009 (0.12)	-0.01 (0.05)	0.85 (0.96)	0.11 (0.04)	0.009 (0.12)	-0.56 (0.29)	0.05 (0.32)
PC ae C36:1	**-0.05 (0.02)**	**0.001 (0.03)**	0.004 (0.02)	0.84 (0.96)	**-0.17 (0.05)**	**0.0002 (0.006)**	**-0.08 (0.02)**	**0.002 (0.03)**	-0.35 (0.29)	0.22 (0.60)
PC ae C36:2	**-0.06 (0.02)**	**<.0001 (0.003)**	0.01 (0.02)	0.51 (0.82)	**-0.27 (0.05)**	**<.0001 (<.0001)**	0.04 (0.04)	0.33 (0.70)	-0.12 (0.29)	0.69 (0.90)
PC ae C38:2	**-0.06 (0.02)**	**0.0002 (0.006)**	0.01 (0.02)	0.75 (0.93)	-0.13 (0.05)	0.005 (0.09)	0.06 (0.04)	0.18 (0.55)	-0.20 (0.30)	0.51 (0.83)
PC ae C38:3	-0.01 (0.02)	0.44 (0.78)	-0.02 (0.02)	0.19 (0.57)	**-0.22 (0.05)**	**<.0001 (0.0003)**	**-0.08 (0.02)**	**0.002 (<0.05)**	-0.49 (0.30)	0.10 (0.41)
PC ae C38:4	0.01 (0.02)	0.50 (0.82)	-0.05 (0.02)	0.03 (0.22)	**-0.17 (0.05)**	**0.0006 (0.02)**	-0.001 (0.05)	0.98 (0.99)	-0.46 (0.32)	0.15 (0.51)
PC ae C40:3	-0.04 (0.02)	0.006 (0.09)	-0.04 (0.02)	0.06 (0.33)	**-0.18 (0.05)**	**<.0001 (0.003)**	0.10 (0.04)	0.02 (0.17)	-0.65 (0.29)	0.03 (0.20)
PC ae C40:4	-0.02 (0.02)	0.34 (0.71)	-0.04 (0.02)	0.06 (0.33)	**-0.16 (0.05)**	**0.002 (0.04)**	0.02 (0.05)	0.72 (0.92)	-0.29 (0.32)	0.34 (0.71)
PC ae C42:2	**-0.06 (0.02)**	**0.0005 (0.01)**	-0.01 (0.02)	0.72 (0.92)	0.02 (0.05)	0.74 (0.92)	0.04 (0.04)	0.40 (0.75)	-0.26 (0.31)	0.41 (0.76)
PC ae C42:3	**-0.05 (0.02)**	**0.002 (0.04)**	0.04 (0.02)	0.03 (0.25)	-0.02 (0.05)	0.67 (0.89)	0.01 (0.05)	0.91 (0.98)	-0.28 (0.31)	0.36 (0.73)
PC ae C42:4	-0.04 (0.02)	0.01 (0.15)	-0.03 (0.02)	0.13 (0.49)	**-0.16 (0.05)**	**0.002 (<0.05)**	-0.01 (0.05)	0.87 (0.97)	-0.24 (0.32)	0.47 (0.80)
PC ae C44:4	**-0.05 (0.02)**	**0.002 (<0.05)**	0.004 (0.02)	0.86 (0.97)	-0.15 (0.05)	0.003 (0.05)	0.04 (0.05)	0.42 (0.77)	-0.41 (0.32)	0.20 (0.57)
SM C16:1	**0.04 (0.01)**	**0.0009 (0.02)**	-0.01 (0.02)	0.77 (0.93)	-0.08 (0.04)	0.08 (0.37)	0.01 (0.04)	0.78 (0.94)	-0.41 (0.27)	0.13 (0.48)
SM(OH) C14:1	-0.02 (0.02)	0.32 (0.69)	0.005 (0.02)	0.79 (0.94)	**-0.20 (0.04)**	**<.0001 (0.0003)**	0.03 (0.04)	0.52 (0.83)	-0.40 (0.28)	0.15 (0.51)
SM(OH) C16:1	-0.01 (0.02)	0.37 (0.73)	-0.01 (0.02)	0.70 (0.91)	**-0.22 (0.04)**	**<.0001 (<.0001)**	0.05 (0.04)	0.26 (0.64)	-0.42 (0.28)	0.14 (0.49)
SM(OH) C22:2	-0.01 (0.01)	0.69 (0.90)	0.01 (0.02)	0.59 (0.86)	**-0.19 (0.04)**	**<.0001 (0.0003)**	0.01 (0.04)	0.84 (0.96)	-0.38 (0.26)	0.15 (0.51)

Analyses were adjusted for sex (in total analysis), age, fasting time, batch, medication use according to ATC code, systolic blood pressure, diastolic blood pressure, HDL cholesterol, triglycerides and glucose levels and consumption of tea or coffee and interactions of sex with each risk factor. Red designates significant associations for women only and blue designates significant associations for men only.

Supplemental table 3. Cross-sectional associations between risk factors (BMI, diet, alcohol consumption, pack-years tobacco and sport) and metabolite-groups. Multilevel modelling with mixed regression models.

	BMI		**Diet**		**Alcohol**		**Pack-years**		**Sport**	
	beta (SE)	**p-value**	**beta (SE)**	**p-value**	**beta (SE)**	**p-value**	**beta (SE)**	**p-value**	**beta (SE)**	**p-value**
Amino acids	-0.01 (0.003)	0.11	0.01 (0.003)	0.13	-0.02 (0.01)	0.04	0.003 (0.01)	0.70	-0.04 (0.06)	0.43
Short-chain acylC	**0.01 (0.002)**	**<0.0001**	-0.001 (0.01)	0.83	0.02 (0.01)	0.06	0.03 (0.01)	0.01	-0.12 (0.08)	0.12
Medium-chain acylC	-0.01 (0.01)	0.39	-0.01 (0.01)	0.35	0.02 (0.02)	0.12	0.04 (0.01)	0.01	-0.11 (0.10)	0.27
Long-chain acylC	-0.01 (0.01)	0.16	0.004 (0.01)	0.54	0.04 (0.01)	0.01	0.02 (0.01)	0.15	-0.08 (0.09)	0.37
PC diacyl	-0.002 (0.003)	0.54	0.002 (0.004)	0.59	0.02 (0.01)	0.02	**0.03 (0.01)**	**0.001**	-0.06 (0.06)	0.32
PC acyl-alkyl	-0.01 (0.004)	0.18	-0.001 (0.004)	0.79	-0.03 (0.01)	0.01	0.003 (0.01)	0.75	-0.10 (0.07)	0.11
SM	-0.004 (0.004)	0.34	-0.002 (0.005)	0.74	-0.01 (0.01)	0.28	0.02 (0.01)	0.07	-0.15 (0.08)	0.05
lysoPC	**-0.02 (0.01)**	**0.0007**	-0.01 (0.01)	0.28	0.001 (0.01)	0.94	-0.03 (0.01)	0.01	0.05 (0.08)	0.54

* Significant associations after multiple test correction are marked in bold type. Significant associations for women only are marked in red.

Supplemental Table 4. Statistically significant longitudinal associations between baseline characteristics (BMI, diet, alcohol consumption, pack-years tobacco and sport) and absolute changes in metabolite values between baseline and follow-up for study participants with changes in risk factors of less than 20% (n=979).

Risk factor	**Metabolite**	**All (N=979)**	
		beta (SE)	**p-value**
BMI	Tyr	0.03 (0.01)	0.0002
	PC ae C34:0	-0.06 (0.01)	<.0001
Alcohol	PC ae C36:2	-0.10 (0.03)	<.0001
Pack-years	PC aa C32:1	0.09 (0.02)	<.0001

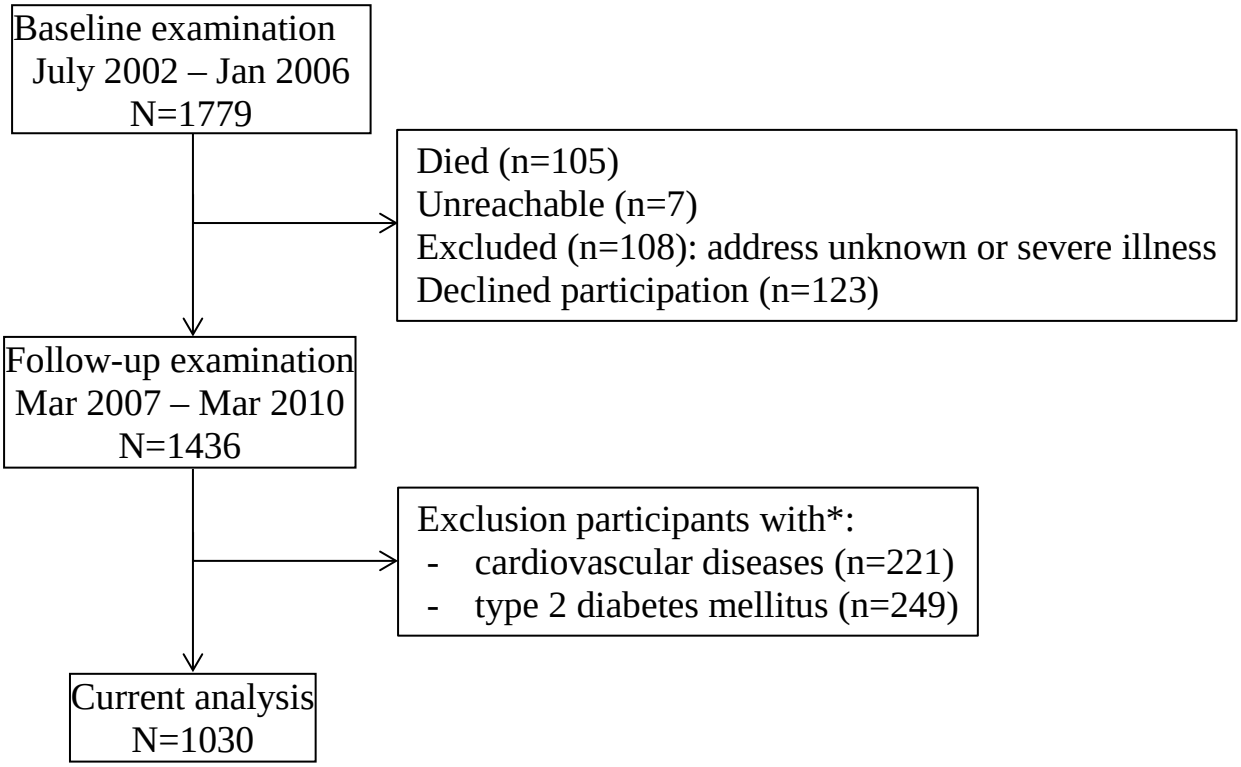
Analyses were adjusted for sex (in total analysis), age, fasting time, batch, medication use according to ATC code, systolic blood pressure, diastolic blood pressure, HDL cholesterol, triglycerides and glucose levels and consumption of tea or coffee and interactions of sex with each risk factor. Significant associations observed only for women are marked in red.

Supplemental Table 5. Cross-sectional and longitudinal associations between baseline lipid levels and insulin resistance and inflammation at baseline or follow-up.

Cross sectional			
Baseline / Baseline	High glucose	High CRP	High RAGE
diacyl PC	↑ **ß=0.001, SE=0.0003, p<0.05**	ß=0.001, SE=0.002, p=0.55	↓ **ß=-3.38, SE=0.98, p<0.001**
acyl-alkyl PC	↓ **ß=-0.001, SE=0.0003, p=0.01**	ß=-0.001, SE=0.002, p=0.74	↑ **ß=2.70, SE=0.90, p<0.003**
lysoPC	ß=-0.0002, SE=0.0006, p=0.77	↓ **ß=-0.03, SE=0.005, p<.0001**	↓ **ß=-4.31, SE=1.96, p<0.03**
Longitudinal			
Follow-up / Baseline	High glucose	High CRP	
diacyl PC	↑ **ß=0.001, SE=0.0003, p<0.01**	**ß=0.005, SE=0.002, p<0.02**	
acyl-alkyl PC	↓ **ß=-0.001, SE=0.0003, p<0.01**	ß=-0.002, SE=0.002, p=0.37	
lysoPC	ß=-0.0003, SE=0.0007, p=0.64	↓ **ß=-0.02, SE=0.005, p<.0001**	

Significant associations are marked in bold type

Supplemental Figure 1. Flow chart of the CARLA study



* 64 participants suffered both

Supplemental Figure 2. Metabotype conservation index. The conservation index of the metabotype of a study participant is defined as the relative rank of the longitudinal intra-correlation of the metabolic profile of that individual compared to the longitudinal inter-correlations with the profiles of all other study participants. In this healthy sub-cohort of the CARLA study (n=1030), 96% of the individuals have a metabotype conservation index of 1.