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Metabolic markers as determinants of future waist-gaining or hip-gaining phenotype in weight-gaining individuals

–

A targeted metabolomics approach in population-based prospective German cohort studies

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Scientific Papers

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Haftenberger M, Mensink G, Herzog B, Kluttig A, Greiser KH, **Merz B**, Nöthlings U, Schlesinger S, Vogt S, Thorand B, Peters A, Ittermann T, Völzke H, Schipf S, Neamat-Allah J, Kühn T, Kaaks R, Boeing H, Bachlechner U, Scheidt-Nave C, Schienkiewitz A. Changes in body weight and obesity status in German adults – results of seven population-based prospective studies. *European Journal of Clinical Nutrition*, 2016; 70(3):300-5.

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Oral presentations

Merz B, Pischon T, Flögel A, Bachlechner U, Nöthlings U, Boeing H. Metabolic status as a determinant for waist-gaining or hip-gaining phenotype in weight-gaining individuals – identifying metabolic constellations using a targeted metabolomics approach in the EPIC-Potsdam study. *10th Annual Meeting of the German Society for Epidemiology,* 2015, Potsdam.

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List of Abbreviations

a	acyl
AA	Amino Acid
AC	Acylcarnitine
BCAA	Branched Chain Amino Acid
BL	Baseline Examination
BMI	Body Mass Index
CDP	Cytidindiphosphat
cm	Centimetre
CI	Confidence Interval
CT	Computer Tomography
CV	Coefficient of Variation
CVD	Cardiovascular Diseases
DEGS	German Health Interview and Examination Survey for Adults
DKFZ	German Cancer Research Center
e	alkyl
EPIC	European Prospective Investigation into Nutrition and Cancer Study
FDR	False Discovery Rate
FFM	Fat Free Mass
FFMI	Fat Free Mass Index
FIA	Flow Injection Analysis
FU	Follow-up Examination
GC	Gas Chromatography
HDL	High Density Lipoprotein
HG	Hip-gaining
IARC	International Agency of Research on Cancer
ICC	Intraclass-Correlation Coefficient
kg	Kilogram

KORA	Cooperative Health Research Platform in the Region Augsburg
LC	Liquid Chromatography
LCL	Lower Confidence Limit
LDL	Low Density Lipoprotein
LoD	Limit of Detection
MONICA	Monitoring of Trends and Determinants of Cardiovascular Disease project in Augsburg
MRI	Magnetic Resonance Imaging
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
OR	Odds Ratio
PC	Phosphatidylcholine
PCA	Principal Component Analysis
PComp	Principal Component
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine N-Methyltransferase
SM	Sphingomyelin
SPComp	Simplified Principal Component
T2D	Type 2 Diabetes
UCL	Upper Confidence Limit
VLDL	Very Low Density Lipoprotein
WG	Waist-gaining
WHO	World Health Organisation
WHR	Waist-to-Hip Ratio
yr	year

1. Introduction

Overweight and obesity, commonly defined as a body mass index (BMI) of more than 25 kg/m² according to criteria of the World Health Organization (WHO) [1], are major public health problems in Germany. According to a representative nationwide health survey, two third of the male and more than half of the female population aged 18-79 years are overweight or obese [2]. Longitudinal data based on this study suggests that the number of overweight and obese individuals may still increase within the next years or stabilizes on a high level, respectively [3].

Overweight is associated with a variety of comorbidities such as hypertension and an increased risk for type 2 diabetes (T2D), cardiovascular disease (CVD) and several types of cancer [4-6]. Thus, overweight and obese people suffer more often from chronic disease conditions and their quality of life is markedly decreased. Based on trend calculations from the WHO dealing with obesity prevalence in Europe on the basis of only a moderate increase till 2020 [7], total expenses on obesity in Germany will increase to at least 25.7 billion € [8]. Thus, obesity is a risk factor with an enormous attributable risk for a lot of associated diseases and high attributable costs.

Body weight gain independent of initial BMI was reported to be an independent risk factor for e.g. T2D [9, 10]. Even on high BMI levels, body weight gain will increase the chronic disease risk even more. As mentioned before, tendencies of a representative nationwide survey showed that prevalence of overweight and obesity will either stabilize on a high level or increase even more [3]. Furthermore, there are partly strong differences in weight-gaining individuals regarding site of body fat accumulation resulting in different body fat distribution phenotypes [11].

A lot of research has been done regarding determinants of body weight gain (including metabolic and hormonal determinants). Especially resting metabolic rate, respiratory quotient as an indirect measure of fat oxidation, insulin sensitivity, inflammatory markers or hormonal levels of e.g. leptin and ghrelin have been shown as being associated with overweight and obesity [12-14]. Those studies were focussing on body weight gain as their primary objective. Within the last years, evidence increased showing body fat distribution to be a very important risk factor for comorbidities [15-17].

So far, the scope of metabolic determinants focused mainly on body weight gain but studies investigating especially future waist or hip gain are lacking, in particular using metabolomics approaches. The present thesis aims to to cover this lack of information.

2. Background

2.1. Definition of overweight and obesity

Obesity is commonly defined as an excess of body fat. Individuals exceeding a BMI of 25 kg/m² are defined as overweight wheras individuals with a BMI above 30 kg/m² are defined as obese [1]. In a policy paper of 2000, the WHO defined obesity as a disease due to its etiological, pathological and pathophysiological aspects [1]. Even the European parliament has requested its member states in 2006 to own the definition of obesity as a chronic disease. Nevertheless, the German health care system did not own this definition yet despite the Federal Social Court gave a verdict in 2003, that obesity *is* a disease based on German health insurance law (BSGE 59, 119 (121)). In Germany, obesity only relates to an excess of body fat whereas most people are aware of the associated conditions.

2.2. Obesity and body fat distribution

An excess deposition of body fat on the trunk/abdomen is called upper body, abdominal or android obesity; whereas an excess deposition of body fat in the gluteofemoral region arround the limbs and buttocks is called lower body, peripheral or gynoid obesity (Figure 1) [18]. The French physician Jean Vague was the first to describe these two different phenotypes of body fat distribution [11]. He noted differences in the distribution of adipose tissue between men and women and implemented the terms ‘android’ to describe abdominal body fat accumulation and ‘gynoid’ for the gluteofemoral body fat accumulation. Furthermore, he was the first to describe divergent associations of those two phenotypes with several diseases like diabetes mellitus, coronary heart disease and gout showing android body fat accumulation to be higher associated with these diseases. As mentioned before, the android phenotype of body fat distribution is reported to be more common in men and the gynoid phenotype to be more common in women. Nonetheless, either phenotype appears within each sex [11].

Figure 1 Schematic illustration of gynoid and android body fat distribution (modified from Vitaleben GmbH [19])

In general, there are two different types of adipose tissue which consists of adipocytes. Visceral adipose tissue is internal abdominal fat and located inside the peritoneal cavity surrounding the internal organs. It accounts for 10-20% of total fat in men and 5-8% of total fat in women and is characterized by rich blood supply through higher vascularization [20]. Subcutaneous adipose tissue accounts for approximately 80% of total body fat mass and is located under the layer of skin [21]. Abdominal obesity is often accompanied by whole body obesity. These two different types of adipose tissue differ in their structure and cell size (between 20 – 200 µm). Adipocytes are the main storage of energy in the human body in form of triglyceride depots. Newly built adipocytes are smaller and have a higher ability of absorbing free fatty acids and triglycerides from the circulatory system. When adipocytes become larger, they become dysfunctional e.g. insulin-resistant and hyperlipolytic [20]. Largest adipocytes can be found in the abdominal area, smallest adipocytes in the gluteofemoral area [20].

Today, the gold-standard to assess the different types of adipose tissue would be the use of Computer Tomography (CT) and Magnetic Resonance Imaging (MRI) [22]. Unfortunately, these methods are very costly and time consuming and therefore impractical to use in large-scaled epidemiological studies. An alternative and the most commonly used measures are waist circumference or waist-to-hip ratio (WHR) to describe abdominal obesity. These measures are easy to perform, cheap to assess and they show good reliability in the assessment of abdominal and visceral adipose tissue [23-26]. There is some evidence that waist circumference is more suitable to assess abdominal obesity than WHR [27]. For waist circumference, increased risk for comorbidities is considered for values above 88 cm for women and 102 cm for men [28, 29].

Body fat distribution is strongly individual and a large variation of this distribution is to be explained by genetic variations [30]. At the end of the last century, the influence of genetics on body fat distribution was discussed and scientists were aware of these impacts. Since then, a lot of genes have been identified that are associated and responsible for the accumulation of adipose tissue in the abdominal or gluteofemoral region. Population-based and twin-studies estimated the influence of genes on the distribution of body fat with 20-60% [30-32]. The other factors mainly influencing this distribution are hormonal. This is obvious, because usually body shape differs noticeable between men and women. On the one hand, in particular oestrogen and prolactin were reported to promote the deposition of fat in the gluteofemoral region of the human body [33]. Age shows as well major impact on body fat distribution. With increasing age, both sexes tend to redistribute their body fat in the

abdominal region due to hormonal changes, in particular decline of oestrogen in women and attenuated production of androgens in men [21].

Obesity-related metabolic consequences were shown to be stronger associated with android phenotype in comparison to gynoid phenotype [34-38]. Abdominal fat accumulation and increased visceral fat is accompanied by unfavourable metabolic changes due to its role as an endocrine organ [13, 21, 39], the impaired metabolic pathways are highly affected. In contrast, higher hip circumference represents gluteofemoral and peripheral adipose tissue and therefore subcutaneous, non-visceral fat [40]. There is strong evidence, that increased waist circumference is associated with an increased risk for the above mentioned diseases [9, 17, 28] but most of these studies neglect the role of hip circumference.

There is evidence, that hip circumference has an important impact on the risk of overall mortality and risk of CVD, diabetes and insulin sensitivity with an adverse effect of low hip circumference and of even inverse risk association of these peripheral fat compartments [15, 34-38, 41].

But not only the single circumferences are of interest, especially the relation of both anthropometric markers to each other is reported to be a key factor of chronic disease risk. The negative effect of abdominal obesity on individual health status should be interpreted with regard to hip circumference [42-44]. Both high waist circumference and low hip circumference have been reported as being associated with CVD risk and Cameron *et al.* [45] reported a serious underestimation of the effect of abdominal obesity on CVD risk when neglecting the effect of hip circumference.

On the other hand, high hip circumference has a suggestive protective effect on chronic disease risk, Heitmann *et al.* [43] reported the necessity to interpret the effects of hip circumference only with regard to waist circumference, BMI or both, too. Hip circumference and BMI are correlated and this may confound the true association between hip and chronic disease risk – resulting in a false positive association of hip circumference and disease risk. It has been reported that high hip circumference attenuates the risk association based on large waist circumference [16, 37] – thus not only the WHR – an established anthropometric marker of risk - but also the difference of these anthropometric markers could be a very interesting risk marker.

2.3. Overweight, obesity and their association to chronic diseases

Overweight and obesity are associated with several chronic diseases e.g. T2D, CVD like myocardial infarction or stroke and several types of cancer [5, 6, 46]. With increasing body fat mass, people are more likely to develop metabolic consequences which result in increased chronic disease risk. A lot of metabolic pathways are affected, e.g. blood lipid metabolism with increased low density lipoprotein (LDL), decreased high density lipoprotein (HDL) and hypertriglyceridemia which is commonly described as dyslipidaemia [47]; impaired glucose metabolism with insulin resistance resulting in increased levels of fasting insulin and blood glucose and chronic low-grade inflammation [48, 49]. Thus, overweight and obese people may develop hypertension, diabetes and atherosclerosis which will put them on high risk for e.g. CVD (Figure 2). Statistics show that 58% of diabetes and 21% of ischemic heart disease are attributable to a BMI above 21 [50].

Furthermore, obesity increases the risk of many types of cancer. Strong evidence exists especially for oesophagus, pancreas, colon and rectum, kidney, thyroid, gallbladder, endometrium and postmenopausal breast cancer [51]. Evidence exists that body weight, weight gain and obesity are responsible for approximately 20% of all cancer cases [6, 52].

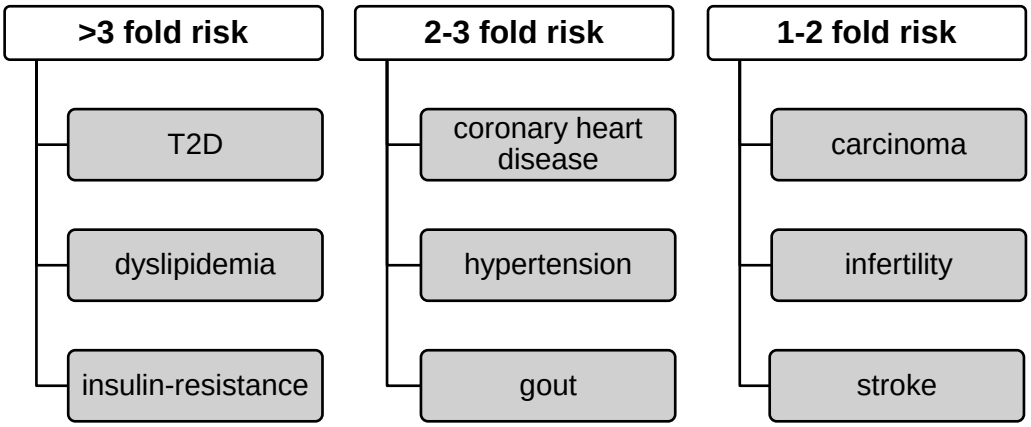

Figure 2 Overview for selected comorbidities and their increased risk due to obesity (BMI≥30) (modified according to WHO, 2000 [1])

Although obesity is clearly associated with metabolic and hormonal disturbances, no single metabolic or hormonal characteristic strongly predicts future weight gain. The literature underscores the complex pathophysiology of obesity and the methodological challenges facing epidemiologic research on metabolic risk factors and weight gain. Current evidence suggests that for most people, body weight gain is probably not a consequence of metabolic defects, but rather a result of many metabolic disturbances caused by a combination of behavioural and environmental factors, such as unhealthy diet and decreased physical

activity [53]. Nonetheless, further research is needed to understand the heterogeneity in metabolic responses to the obesogenic environment.

There are numerous studies reporting distribution of body fat as a more important factor regarding elevated risk for those chronic diseases than weight gain [15-17]. In the 1980s, major progress was made in terms of metabolic implications of different body shapes in pathophysiological pathways. Evidence of increased risk for CVD and T2D could be strengthened. Additionally, impaired glucose tolerance, insulin resistance and fasting hyperinsulinemia where pointed out as the underlying pathophysiological process. Hypertriglyceridemia, hypercholesterinaemia and lower HDL cholesterol accompanied with hypertension and increased risk for stroke and coronary heart disease were reported for people that tend to have a large waist circumference [48, 54-58].

2.4. Causes of obesity

The causes of obesity are multifactorial and cover endogenic and exogenic factors (Figure 3). Besides family history (genetic disposition) or health-related causes (e.g. Cushing syndrome, hypothyroidism), obesity and substantial weight gain is mostly explained with sedentary lifestyle factors like hypercaloric diet and physical inactivity [53]. Indeed, it is of interest whether people tend to gain weight at their waist or at their hip due to different associations with chronic disease risk.

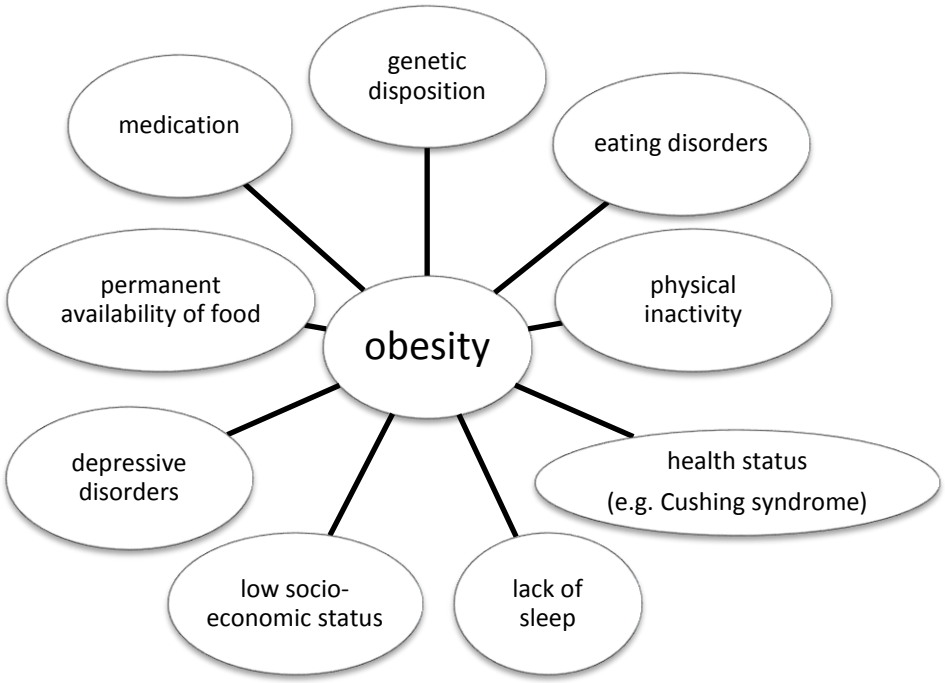

Figure 3 Selected causes of obesity (modified from DAG e.V. guidelines 2014 [59])

As mentioned before, obesity has major impact on the human metabolism and especially abdominal obesity is likely to affect several biochemical pathways due to the role of visceral adipose tissue as an endocrine organ. A lot of research has been done to identify and describe the relation of metabolic and hormonal determinants and predictors of body weight gain - thus in the development of obesity. A wide range has been covered including pro- and anti-inflammatory markers [13, 60, 61], liver enzymes, hormones and especially markers of insulin sensitivity [12, 62, 63]. There is no clear evidence that these factors are likely to favour weight gain and a lot of studies are restricted to specific populations and age groups. A reason could be the metabolic heterogeneity between populations, so results are not generalisable.

Metabolic profiles are depending on three major influencing factors: age, sex and ethnicity [40]. Except of age, those factors are genetically given just like body fat distribution.

In these strongly varying profiles, the identification of biomarkers in terms of risk prediction is of major interest (metabolic biomarkers for predicting cardiovascular disease) [64, 65]. Single biomarker epidemiology is highly specific and sensitive, but on cost of a narrowed view on the metabolism. A possibility to get a deeper inside is given within the field of systems biology [66]. Systems biology is the study of biological systems that cannot easily be reduced to the sum of its single components. Therefore systems biology tries to understand organisms as a whole apart from single biomarker investigation. The science of metabolomics is one part of this systems biology approach and nowadays metabolic status can be efficiently determined by metabolomics.

2.5. Metabolomics

Metabolomics is the fourth ‘omics’ research field besides genomics, transcriptomics and proteomics. Genomics covers the entity of all genes (about 25,000), transcriptomics is dealing with translational factors (about 10,000 mRNA), proteomics is especially dealing with enzymes and their activities (about 1,000,000 proteins) and last but not least the study of metabolomics which is about the entity of the human metabolome with all molecules below 1500 Dalton (about 2500 metabolites) (Figure 4) [64, 67]. Within this four ‘omics’ research fields, the central dogma of molecular biology describes the information flow from genomic information (DNA) through mRNA transcripts, which are then translated to proteins and enzymes. These enzymes are directly influencing the concentrations of their substrates and products, which are integrated in tightly controlled metabolic pathways. Especially within subclasses of metabolites, different molecules may appear in the same metabolic pathways and could be easily transformed into one another via enzyme activities [68]. Therefore, from genomics to metabolomics, the knowledge is shifting from genotype to phenotype [69] according the central dogma of molecular biology [68].

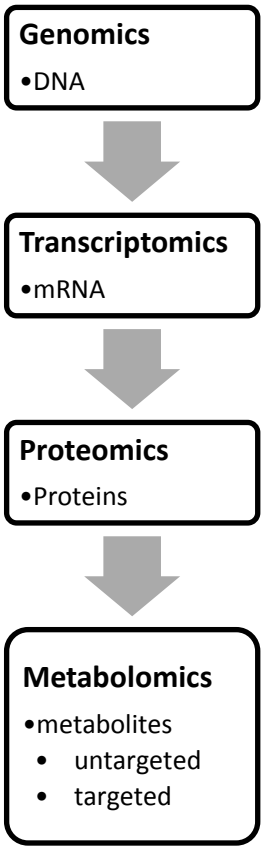

Figure 4 Overview on the 'Omics' sciences and their field of research

The study of metabolomics can be described as the study of metabolic profiling through systematic analysis of substrates and products of all biochemical pathways and aims to create snapshots of the human metabolome [67]. Metabolic profiling can be performed in nearly all biological samples, e.g. serum, plasma, urine or tissue samples. The comparison of results should be restricted on the same source of samples because concentration ranges of the chosen metabolites vary considerably in different tissues [70]. Within the field of metabolomics, we differentiate between targeted and untargeted approaches.

2.5.1. Untargeted metabolomics

Untargeted metabolomics aims to comprehensively measure all measurable metabolites of a sample and is not restricted to subclasses or specific pathways. Identification of many unknown metabolites after quantification is usually necessary, but this provides the opportunity of discovering novel metabolite species and pathways [71]. A

common technique of untargeted metabolomics measurements includes the use of nuclear magnetic resonance (NMR) spectroscopy, but gas chromatography (GC) or liquid chromatography (LC) coupled with mass spectroscopy (MS) are used, too. NMR is able to detect a wide spectrum of metabolites. It is less sensitive than MS, but sensitive enough to identify metabolites in measurements of unmodified biological fluids. There is no necessity of an invasive and destructive sample preparation to make the metabolites measurable and is therefore well-suited for the use in untargeted metabolomics [66].

2.5.2. Targeted metabolomics

The scope of targeted metabolomics is narrowed and quantifies pre-defined groups of chemically characterized metabolites - a subset of the metabolome [67]. This approach has the advantage that all quantified metabolites are already identified; results are more robust through the use of internal standards and show better comparability among each other. So, extensive understanding of physiological processes is possible on the basis of covered metabolic processes, enzyme kinetics and established pathways [67]. In comparison to untargeted metabolomics, data quality is improving at the expense of a narrowed view on the metabolome [71]. MS coupled with LC or GC (LC-MS and GC-MS) are often used to quantify metabolites in targeted metabolomics measurements. A key advantage of this method is its high sensitivity but MS requires the metabolites to be separated from the biological sample before detection and chemically modified to make them more volatile, especially if GC-MS is used. This treatment may damage unidentified metabolites and might give misleading results in untargeted metabolomics. So this method is more common within the field of targeted metabolomics where the quantification is based on already identified metabolites [66].

The platform reproducibility is a challenge to any targeted metabolomics experiment. Contributions from three sources of variance should be considered: system variance, variance introduced during sample preparation and the intra- and inter-individual biological variance. These confounders can be evaluated using a number of approaches [67]. Biological variance can be addressed using the coefficient of variation (CV). Either blood/serum or reference samples are measured multiple times on each plate or on several plates to get within-plate or between-plate variance, respectively. The standard deviation for those measures is divided by the mean value of the dataset for each metabolite to obtain the CV which is used with study-specific cut-offs as quality-control criteria.

2.5.3. Opportunities of metabolomics in obesity research

The study of metabolomics is challenging in many ways. There are many factors influencing the human metabolome. Besides intrinsic factors like e.g. age, sex, genetics, health status and resting metabolic rate [40, 72-74] as well extrinsic factors like diet, physical activity, smoking, alcohol consumption and environment show identifiable impact on the human metabolome (Figure 5) [75-79]. All this influencing factors lead to metabolic phenotypes and individual metabolic profiles that can be measured using metabolomics approaches.

The discovery that altered metabolite profiles are caused by certain diseases or by drug effects came up in the mid-1980s [66]. Since then, targeted metabolomics approaches have already been used to identify specific phenotypes that are prone to develop a disease of interest, e.g. T2D [65, 80-82] or identified altered metabolic profiles in individuals with prevalent diseases e.g. Parkinson´s disease, CVD or cancer [83-86].

Furthermore, targeted metabolomics approaches have been investigated regarding their association to markers of body composition or anthropometric markers, respectively [87-91], to identify metabolic phenotypes that are associated with specific characteristics such as fat free mass (FFM). As described before, two different phenotypes of body fat distribution appear in men and women, differing in their corresponding risk for chronic diseases. The study of metabolomics was already applied to link metabolic profiles with disease risk in general but up to now, no study is published investigating the role of metabolic markers measured with a metabolomics approach as determinants of one of the body fat distribution phenotypes.

2.6. Objectives

Because body fat distribution has major impact on the risk association of several chronic diseases with abdominal obesity and the android body fat distribution having worsening impacts, there are several questions that were addressed in the present thesis (Figure 5).

1) The first part aims to describe changes of anthropometric markers to assess tendencies to a specific body fat distribution in weight-gaining individuals using data of three independent representative prospective German cohort studies.
2) Based on these results, the second part aims to identify single metabolites that are favouring the development of either waist-gaining or a hip-gaining phenotype in weight-gaining men and women using data of two independent prospective German cohort studies.
3) The third part of this thesis aims to compare a single metabolite and a principal component approach to investigate associations of targeted metabolomics profiles with either waist-gaining or a hip-gaining phenotype using data of one prospective German cohort study to address the highly complex metabolomics data in two different approaches and strengthen the evidence of observed results.

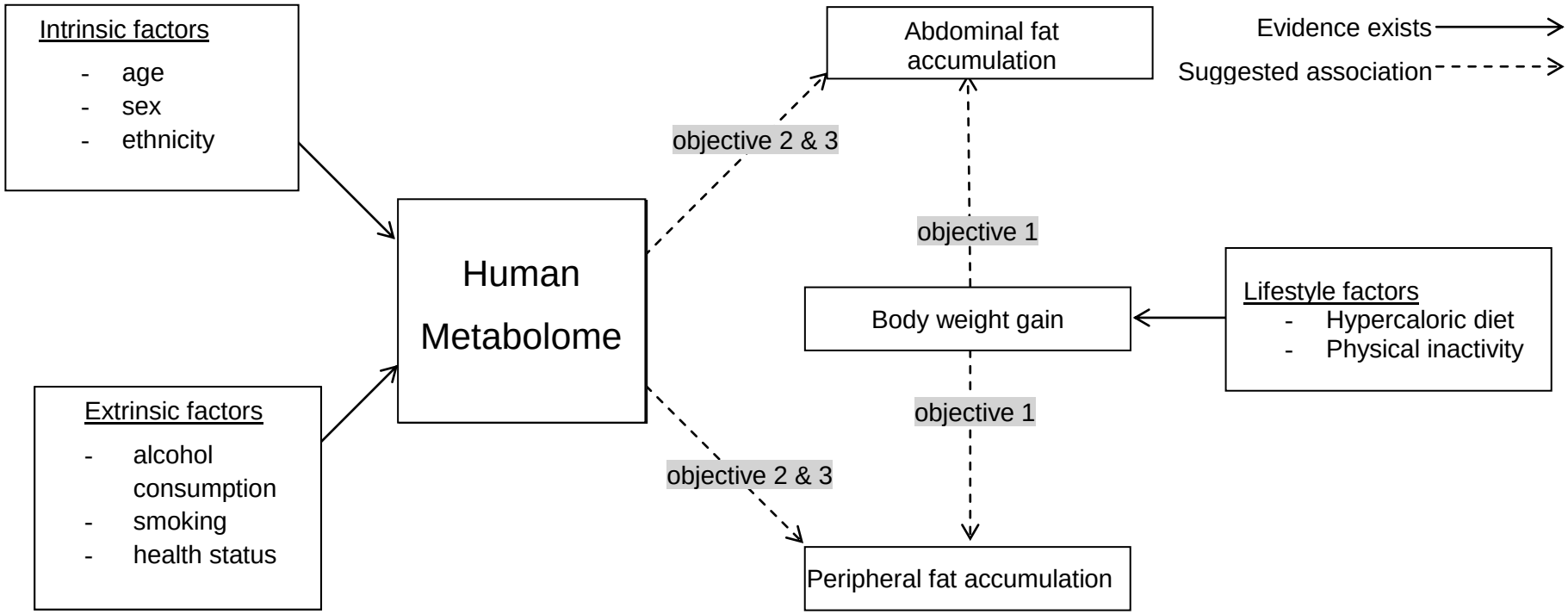

Figure 5 Objectives of this thesis in context to human metabolome, body fat accumulation and influencing factors

3. Subjects and Methods

3.1. Study design

The thesis is based on data of three prospective German cohort studies. Each step and statistical model of the following analyses was first tested and implemented in the European Prospective Investigation into Cancer and Nutrition (EPIC) Potsdam study. A standardized analysis plan based on the EPIC-Potsdam implementations was provided to the corresponding cohorts. All results were collected for further meta-analytical analyses and interpretation. No data pooling was done.

3.1.1. European Prospective Investigation into Cancer and Nutrition Study

The EPIC study is one of the largest ongoing multicentre cohort studies in the world with more than half a million (521,000) participants recruited across 10 countries all over Europe and coordinated by the International Agency for Research on Cancer (IARC) of the WHO in Lyon, France [92]. For Germany, two study centres at the German Cancer Research Center (DKFZ) in Heidelberg with 25,540 participants and at the German Institute of Human Nutrition (DIfE) in Potsdam with 27,548 participants are part of this large multi-centre cohort study [93, 94]. The primary aim of this cohort study was to study the associations between diet and cancer, but other risk factors and chronic diseases were considered additionally.

For EPIC in Potsdam, the study region included the city of Potsdam and surrounding small to middle-town cities and communities. Participants mainly in the age of 35 (women) or 40 (men) to 65 years from the study region were recruited between 1994 and 1998 based on general population registries, but interested people that did not match the age criteria were also allowed to be part of the study [94]. The participation rate for baseline examination was 27%, participants tended to be more health conscious and higher educated than the general population [94]. For the present thesis, data from the fourth follow-up examination conducted between 2004 and 2008 was used. The study population of EPIC-Potsdam was biannually contacted by mail for follow-up information. Of this study population, a subcohort of 2,500 participants aged 20-68 years at time of recruitment was randomly selected for specific biomarker measurement using a case-cohort design. The results of this subcohort are expected to be generalisable through the use of random selection and are therefore representative for the full EPIC cohort in Potsdam [95]. The EPIC-Potsdam study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Medical Society of the State of Brandenburg. Individuals provided written informed consent prior to participation of the study

3.1.2. Cooperative Health Research Platform in the Region Augsburg Study

The Cooperative Health Research Platform in the Region Augsburg, Bavaria (KORA) is a regional research platform for population-based surveys and subsequent follow-up studies and was designed to continue and expand the MONICA (Monitoring of Trends and Determinants of Cardiovascular Disease) project in Augsburg [96]. The study was approved by the local Ethic Committee of the Bavarian Medical Association.

Individuals with German nationality were sampled in a two-step sampling procedure: A cluster sampling procedure was performed in Augsburg and 16 communities followed by a stratified random sampling in each community. Beginning in 1984, four cross-sectional studies were performed in a 5-year interval with follow-up studies for third and fourth survey conducted 7-10 years later from baseline examination. The fourth KORA survey (S4) was conducted between 1999 and 2001 with a 7-year follow-up period and follow-up examination between 2006 and 2008. The fourth survey consists of 4,261 participants of which 3,080 individuals aged 25 to 74 also participated in the follow-up examination [97], of which again 1,614 individuals aged 55 and older were selected as a subsample for targeted metabolomics measurement [87].

In all surveys, all participants were examined by trained medical staff including anthropometric and blood pressure measurements according to standardized operating procedures. Furthermore, standardized interviews on sociodemographic variables, several risk factors including e.g. smoking, alcohol consumption and physical activity, medical history (personal and family), medication use, and even more were performed. Each study was conducted according to the guidelines laid down in the Declaration of Helsinki and all participants gave written informed consent before participation on the study.

The primary aim of the KORA study was to study risk factors of CVD due to its origin of the MONICA project, but with growing knowledge of associated diseases and pathophysiological processes, research topics have become broader. The KORA S4 is dealing with the topics of e.g. CVD, diabetes, obesity, dermatology and allergy [96].

3.1.3. National health interview and examination survey

The German Health Interview and Examination Survey for Adults (DEGS) is part of the health monitoring at the Robert Koch-Institute (RKI) and was primarily designed as a periodically survey [98]. Study participation required permanent residence in Germany according to local population registries. To establish a longitudinal study within DEGS, participants from the survey conducted in 1998 (N=7,124) were reinvited to participate in 2008-2011. Between 2008 and 2011, 3,959 participants from the German National Health Interview and Examination Survey 1998 (GNHIES98) (response rate 62 %) of whom 914 were surveyed by means of interviewed only and 3,045 were both interviewed and examined [3].

Data collection included several questionnaires and (computer-assisted) personal interviews on e.g. habitual diet, assessment of current medication and medical history including family history. Furthermore, collection of biomaterial (urine and blood) and standardized physical performance tests were conducted [98].

Aims of the study were to analyse current health status, changes in health status, health risks and resources, functional capacity levels and disability in the adult German population. A major study objective was to estimate the prevalence of diseases and risk factors with high public health impact and the changes over time [98].

The implementation of DEGS conformed to the guidelines of the Declaration of Helsinki [99] and to the German Federal Data Protection Act. The DEGS study protocol was consented with the Federal and State Commissioners for Data Protection and approved by the Charité-Universitätsmedizin Berlin Ethics Committee. Individuals provided written informed consent prior to participation of the study [98].

3.2. Study population

The case-cohort subsample for specific biomarker measurement in EPIC-Potsdam consists of 2,500 participants from the overall EPIC-Potsdam study population [95]. The KORA S4 cohort consists of 4,261 participants, of which 3,080 individuals also participated in a follow-up examination F4 [97]. In a subsample of 1,614 participants aged 54 to 75 targeted metabolomics measurement was performed [87]. The national survey DEGS provided data of 3,045 participants who were both interviewed and examined [3]. Therefore, the investigated studies included a total of 7,159 participants (2,500 in EPIC-Potsdam, 1,614 in KORA, 3,045 in DEGS). Of these participants, 829 individuals with missing information for variables of interest at baseline or follow-up (299 in EPIC-Potsdam, 496 in KORA, 34 in DEGS) and 14 pregnant or postpartum women (DEGS) were excluded. Because of the particular interest in specific weight gain phenotypes, 2,193 individuals without body weight gain in the follow-up period (557 in EPIC-Potsdam, 526 in KORA, 1,110 in DEGS) were also excluded resulting in a final analytical study sample of 4,126 individuals (1,644 in EPIC-Potsdam, 592 in KORA, 1,890 in DEGS), 1,802 men and 2,324 women (Figure 5).

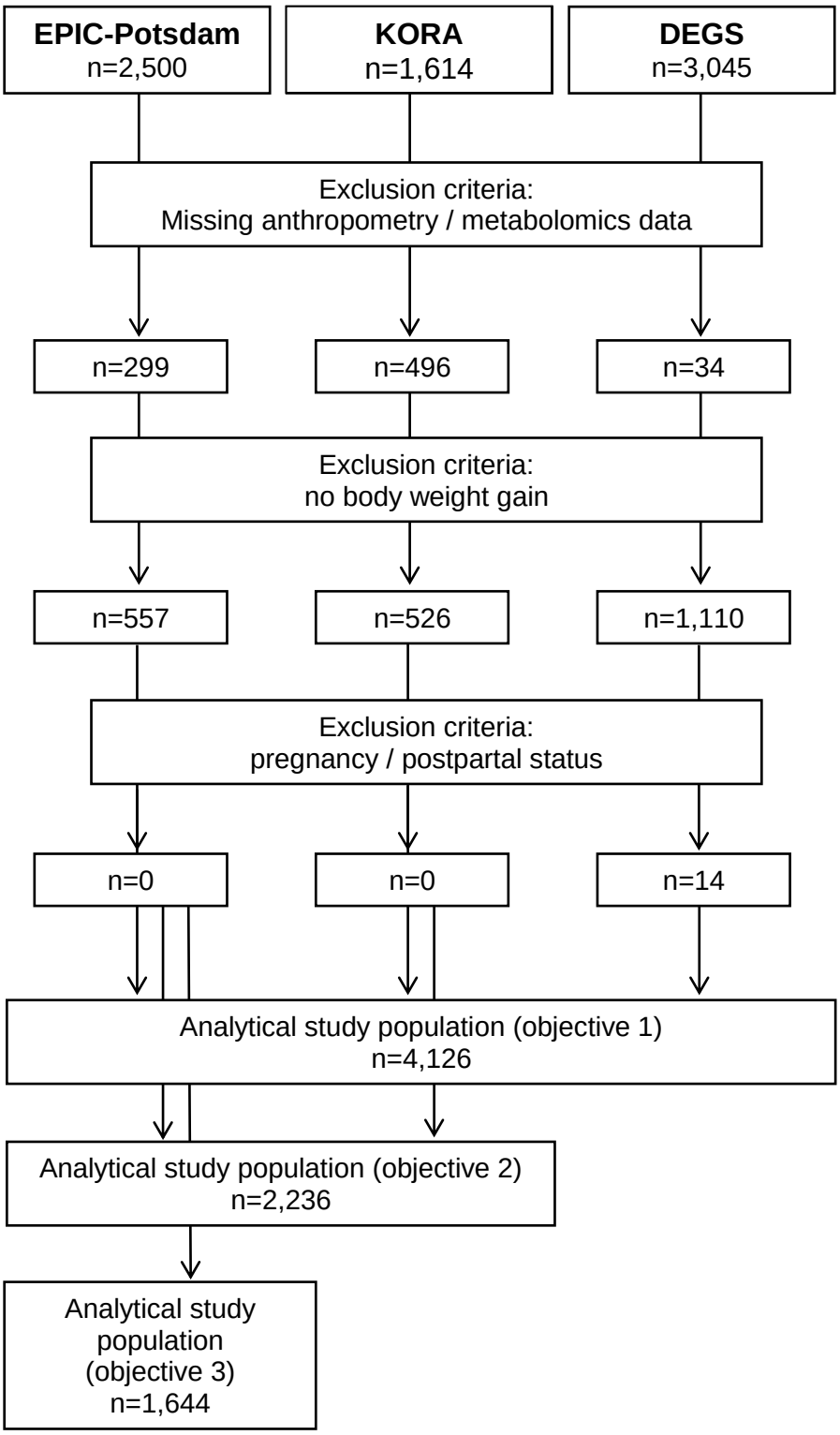

Figure 6 Flow chart illustrating the exclusion criteria in terms of analytical study population

3.3. Tendencies to a specific body fat distribution in weight-gaining individuals in Germany (objective 1)

3.3.1. Study population

Data from all three population-based prospective German cohort studies (EPIC-Potsdam, KORA, DEGS) were included in this part of the thesis. Thus, the final analytical study population consists of 4,126 participants, 1,802 men and 2,324 women.

3.3.1. Variable assessment at baseline

For baseline examination, participants were invited to the study centres and examined by trained staff according to study-specific standardized procedures in a standing position using a non-elastic flexible tape. For EPIC-Potsdam, all baseline measures were performed in underwear without shoes, for KORA and DEGS in light clothing without shoes. In EPIC-Potsdam, waist circumference (in cm) was measured at the midpoint between the lowest rib and the superior border of the iliac crest and on the narrowest point for KORA and DEGS. Hip circumference (in cm) was measured at the most sweeping point of the buttocks horizontal around the body in EPIC-Potsdam and at the most protrusion of the gluteal region between the superior border of the iliac crest and crotch in KORA and DEGS.

For follow-up examination in EPIC-Potsdam, information on body weight, waist and hip circumference was collected as self-report. Participants received a letter with a standardized non-elastic flexible tape and a written instruction. With regard to reduce bias due to measurement error and underreporting, self-reported values were corrected using EPIC-specific equations developed to correct self-reports [100, 101]. For follow-up examination in study centres of DEGS, slight modification in standardized procedures was done measuring participants in underwear instead of light clothing at baseline. No modifications were done in KORA.

For body weight, waist and hip circumference, average annual changes were calculated by subtracting the baseline measure from the follow-up measure divided by the individual follow-up time in years. The difference of average annual changes in waist circumference minus hip circumference was calculated for every individual. Negative values identified people gaining more hip circumference, positive values identified people gaining more waist circumference.

Information on age, sex, smoking status (current, former, never smoker), alcohol consumption (in g/d) and physical activity (by sports) were assessed with study-specific

socio-demographic and lifestyle questionnaires. Four categories were formed in each cohort with available information on regular physical activity: no sports, less than 1h/week, 1-2 h/week and more than 2h/week. Supplementary, a women-specific questionnaire assessed information regarding menopausal status und parity [93, 96, 98].

Menopausal status was defined 2-categorical as pre- and postmenopausal. In EPIC-Potsdam, postmenopausal was defined as suspended menses (non-surgical induced) for more than 1 year and no hormone replace therapy (HRT) or a started HRT during the last menses. In KORA, postmenopausal was defined as absence of menses; all other women were categorised as premenopausal. In DEGS, postmenopausal status included women with absence of menses for more than 1 year or HRT. All other women were categorised as premenopausal.

Information on chronic disease status including myocardial infarction, stroke, any type of diabetes and malignant types of cancer was assessed via standardized questionnaires in each study. For EPIC-Potsdam, information on the diseases of interest included prevalent diseases at baseline examination or incident diseases within the follow-up period. For KORA, prevalent or incident disease status included the period from 1 year before baseline examination to the follow-up period. For DEGS, information on disease status refers to ever suffered from the disease of interest.

3.3.2. Statistical analysis

Self-reported measures of body weight, waist and hip circumference for follow-up in EPIC-Potsdam were corrected using equations from Spencer *et al.* (Figure 7). All values and analyses in this thesis are based on the corrected measures.

body weight

men $\text{body weight}_{corr} = \mathbf{0.561} + (\mathbf{1.012} * \text{body weight}_{SR}) + (\mathbf{0.006} * \text{age}_{FU})$

women $\text{body weight}_{corr} = \mathbf{0.444} + (\mathbf{1.010} * \text{body weight}_{SR}) + (\mathbf{0.006} * \text{age}_{FU})$

waist circumference (waist)

men $\text{waist}_{corr} = \mathbf{7.791} + (\mathbf{0.972} * \text{waist}_{SR}) - (\mathbf{0.035} * \text{age}_{FU})$

women $\text{waist}_{corr} = \mathbf{9.022} + (\mathbf{0.847} * \text{waist}_{SR}) + (\mathbf{0.091} * \text{age}_{FU})$

hip circumference (hip)

men $\text{hip}_{corr} = \mathbf{42.812} + (\mathbf{0.637} * \text{hip}_{SR}) - (\mathbf{0.075} * \text{age}_{FU})$

women $\text{hip}_{corr} = \mathbf{20.040} + (\mathbf{0.818} * \text{hip}_{SR}) - (\mathbf{0.011} * \text{age}_{FU})$

Figure 7 EPIC-specific equations to correct self-reported body measures [96, 97]

Abbreviations: corr=corrected, FU=follow-up, SR=self-reported
Units: age (yr), body weight (kg), waist and hip circumference (cm)

To investigate and describe longitudinal changes of anthropometric markers and with regard to different follow-up periods for each study, average annual changes in body weight, waist and hip circumference were calculated (Figure 8).

$$\text{average annual weight change (kg/yr)} = \frac{\text{body weight FU - body weight BL}}{\text{FU time}}$$

$$\text{average annual waist change (cm/yr)} = \frac{\text{waist circumference FU - waist circumference BL}}{\text{FU time}}$$

$$\text{average annual hip change (cm/yr)} = \frac{\text{hip circumference FU - hip circumference BL}}{\text{FU time}}$$

Figure 8 Calculation of average annual changes of anthropometric markers

Abbreviations: BL, Baseline examination; FU, follow-up;
Units: age (yr), body weight (kg), waist and hip circumference (cm)

Because of the particular interest in weight-gaining individuals, all individuals with an average annual weight change less than or equal to zero were excluded from all analyses of this thesis. To describe the general tendencies of changes in body measures in weight-gaining individuals, the difference of average annual changes of waist and hip circumference was calculated by subtracting average annual changes of hip and waist circumferences (Equation 1). Negative values identified people increasing rather hip circumference, positive values identified people increasing rather waist circumference.

Equation 1 Calculation of the average annual waist-hip difference

average annual waist-hip difference = average annual waist change – average annual hip change

Unit: average annual changes (cm/yr)

Descriptive data is presented as mean and standard deviation (SD) for continuous and absolute quantities and percentage for categorical variables. Depending on their variance and cell counts, paired Student's t-test or sign-test for continuous and χ^2 or Fishers exact test were applied to test for statistically significant differences between groups for continuous or categorical variables, respectively. According to criteria of the WHO [1] the BMI (in kg/m²) was calculated as body weight divided by height (in m) squared and individuals were classified in overweight (BMI ≥ 25 kg/m²), pre-obesity (25 ≤ BMI < 30 kg/m²) and obesity (BMI ≥ 30 kg/m²). Individuals were categorised as abdominally obese if men exceeded a waist circumference of 102 cm and women of 88 cm [28, 29]. Of the previously defined waist-hip difference, Student's t-test was applied to test for differences between sexes. Furthermore, the relationship between this difference and average annual weight change was investigated in each study using Pearson correlation for men and women separately. To the current knowledge, no other study applied this average annual waist-hip difference yet, thus relationship between established anthropometric markers including waist and hip circumference, BMI, WHR and waist-hip difference was investigated in EPIC-Potsdam using Pearson correlation coefficients.

In the nationwide study DEGS, anthropometric markers assessed in the study were additionally corrected for deviations of the sample with regard to age, sex, region, nationality, type of municipality and education from the population structure as of December 31st, 1997 [102].

To investigate the potential bias in the present analysis due to drop-out in the investigated cohorts, study-specific probabilities for reparticipation at follow-up examination were calculated in DEGS with the lowest response rate of 43% [3] and EPIC-Potsdam with

the highest response rate 92% [103]. Multivariable logistic regression models with response at follow-up as the dependent and potential predictors of drop-out as independent variables were used for the calculation of the probability of reparticipation. As long as assessed at the baseline examination, sociodemographic variables including age, sex, education and income, BMI, lifestyle variables including smoking, alcohol consumption and physical activity were considered as independent variables. Individuals that died within the follow-up period were not treated as drop-out and therefore not considered in the calculation. The calculated probabilities of reparticipation were then applied in a sensitivity analysis as inverse-probability weights [104]. Again, the weighting factor for DEGS included additionally corrected deviations of the demographic population structure as of December 31st, 1997 [102]. Calculations in all studies were done using statistical software SAS release 9.4 (SAS Institute Inc, Cary, NC, USA).

3.4. Identification of phenotype associated metabolites (objective 2)

3.4.1. Study population

Participants from EPIC-Potsdam and KORA were involved in this part of the thesis. 1,644 weight-gaining individuals of EPIC-Potsdam and 592 weight-gaining individuals of KORA resulted in a total analytical study population of 2,236 participants, 918 men and 1,318 women.

3.4.2. Blood sample collection

During their stay at the EPIC-Potsdam study centre for baseline examination, a total of 95.7% of the participants in Potsdam provided a 30ml blood sample which was drawn by trained medical staff. For baseline examination appointments before noon, participants were informed about the blood sampling and advised not to eat anything before their examination. All in all, the proportion of fasting blood collections was 28% [94]. Of the 30ml venous blood, 20ml were inserted into citrate containing monovettes, other 10ml into monovettes without any anticoagulant and immediately fractioned into serum, plasma, buffy coat and erythrocytes. During withdrawal and processing exact time and room temperature were steadily documented. According to a standardized protocol, the samples were aliquoted into straws of 0.5ml resulting in 12 straws of plasma, 8 straws of serum, 4 straws of buffy coat and 4 straws of erythrocytes up to a total of 28 straws for each participant. This straws were then stored at -196°C in tanks of liquid nitrogen [93].

In KORA, fasting serum samples for metabolic analysis were collected during study centre visits. The blood drawing occurred after a period of overnight-fasting (minimum of 8 hours) using S-Monovette^{TM} serum tubes (SARSTEDT AG & Co., Nümbrecht, Germany). Tubes were inverted two to three times, spent five minutes on the universal shaker (SARSTEDT AG & Co., Nümbrecht, Germany) before being allowed to rest for 40 minutes at 4°C for total coagulation. Later on, tubes were centrifuged for 15 minutes at 2,660g, serum was separated and filled into synthetic straws which were stored in liquid nitrogen (-196°C) until analysis [105].

3.4.3. Sample preparation and metabolomics measurement

The measurements for EPIC-Potsdam and KORA were performed in the Genome Analysis Center at the Helmholtz Center Munich. Different high-throughput targeted metabolomics measurement Absolute*IDQ*™ kits (Biocrates Life Sciences AG, Innsbruck, Austria) were used in the studies, but sample preparation and measurement procedures were equivalent.

In EPIC-Potsdam, targeted metabolomics Absolute*IDQ*™ p150 kit was used to quantify serum metabolites. The p150 kit uses a flow injection analysis mass spectroscopy coupled with multiple reaction monitoring scans (FIA-MS/MS) technique on an API 4000™ triple quadrupole mass spectrometer (ABSciex Deutschland GmbH, Darmstadt, Germany) equipped with an electrospray ionization source for the detection of metabolites [70].

Sample preparation was done according to a Biocrates provided protocol and has been previously described in detail [70]. In brief, after centrifugation 10µl of each serum sample were pipetted by an automated Hamilton ML Star robotics system (Hamilton Bonaduz AG, Bonaduz, Switzerland) on special double-filter 96-well plates which contained already isotope labelled non-radioactive internal standards, blank samples and quality controls. After incubation, the filters were dried under nitrogen stream and amino acids (AA) were derivated with 5% phenylisothiocyanate reagent. Then the filters were dried again followed by the extraction of metabolites and internal standards with 5 mmol/l ammonium acetate in methanol, centrifuged through a filter membrane and then diluted with a MS running solvent. For all measurements, two 20µl injections (for positive and for negative electrospray ionization mode) were applied by the standard flow injection method [70]. Specific analytical software Met*IDQ*™ (an integral part of the kit) provided by the manufacturer Biocrates Life Sciences AG was used for calculation of concentrations and evaluation. This software compares measured metabolites in a defined extracted ion count section to specific labelled internal standards or non-labelled non-physiological standards (semi-quantitative) of the 96-well plate. This method is in conformance with the “Guidance for Industry – Bioanalytical Method Validation” of the Food and Drug Administration, which implies the proof of reproducibility within a given error range [70]. Limits of Detection (LoD) were set to three times the values of the manufacturer provided zero samples (methanol and 10 mmol/l phosphate buffer). Lower and upper limit of quantification were determined experimentally by the manufacturer.

For KORA, targeted metabolomics Absolute*IDQ*™ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria) was used to quantify serum metabolites. The p180 kit is an extension

of the p150 kit and uses the combination of FIA-MS/MS and Liquid Chromatography mass spectroscopy (LC-MS) on the mass spectrometer equipped with an electrospray ionization source for the detection of metabolites [70].

Sample preparation was equivalent to EPIC-Potsdam described afore. Specific for the p180 kit, the plate was centrifuged at 100g for 2 min, with plate 1 (FIA plate) receiving about 250µl sample. After removal of the upper plate, 150µl of each sample were transferred onto the LC-MS plate with additionally 150µl HPLC water. 500µl of a Biocrates solvent diluted in methanol as MS running solvent were added to the FIA plate. The LC-MS plate was measured first by scheduled multiple reaction monitoring, the FIA plate was stored at 4° and then measured by FIA equivalent to EPIC-Potsdam. Analogue to the p150 procedure concentrations were calculated and evaluated using the Met*IQ*^{TM} software of Biocrates Life Sciences AG. All concentrations are were calculated in mmol/l [106].

These targeted metabolomics approaches simultaneously identified and quantified 163 metabolites (p150) or 186 metabolites (p180), including AA, acylcarnitines (AC), different kinds of glycerophospholipids (lyso-, diacyl-, and acyl-alkyl-phosphatidylcholines (PC)), sphingomyelins (SM) and hexose (H) (sum of six-carbon monosaccharides without distinction of isomers) and exclusively for p180 biogenic amines. The manufacturer selected the metabolites based on the robustness of their measurements. The uncertainty of the measurements was 10% for most of the metabolites. Regarding accuracy, all included metabolites were found in the range of 80–115% of their theoretical values [107].

AAs were presented according to three letter abbreviations. Lipid side chains were abbreviated as Cx:y with x referring to the number of carbons in the side chain and y to the number of double bonds. PCs were additionally differentiated based on their type of bond to glycerol with ‘a’ for acyl and ‘e’ for alkyl bond, the prefix ‘lyso’ indicated only a single fatty acid side chain. Diacyl- and acyl-alkyl-PCs contain two fatty acid side chains, for example “PC ae C40:1” denotes a phosphatidylcholine (PC) with an acyl bond (a) and an alkyl bond (e) side chain. This metabolite contains 40 carbons in both side chains and a single double bond in one of them. Unfortunately these methods could not determine the exact distribution of carbons in each bonded fatty acid or the precise position of double bonds within the molecule. All ACs were derivates of free L-carnitine (C0) linked to one fatty acid [105].

Within the p180 kit, leucine and isoleucine were quantified separately whereas the p150 kit quantified these metabolites combined as xleucine. For the present analyses, these measures were not considered because of potential heterogeneity.

For the selection of the final metabolomics dataset, EPIC-Potsdam and KORA used slightly different quality criteria. Serum or reference samples were measured multiple times

to get within-plate or between-plate variance, respectively. The standard deviation for those measures was divided by the mean value of the dataset for each metabolite to obtain the CV as a common measure of technical variation [87, 108].

In EPIC-Potsdam, metabolites with a too large variation given by a CV (within- or between-plate) of greater than 50% or with the majority of values below the LoD were excluded from the analyses. The median analytical variance of EPIC-Potsdam samples was a 7.3% within-plate coefficient of variation and a 11.3% between-plates coefficient of variation [108].

For KORA, metabolites with more than 5% missing values (1 metabolite) and a CV of greater than 25% (n=11) were excluded from the analysis. Metabolite concentrations were defined as outliers if values exceeded a range of mean +/- five standard deviations. In that case concentrations were treated as missing values and imputed using R package ‘mice’. Individuals with more than three metabolites exceeding this range were excluded from the analyses [87].

After study specific quality criteria, a total of 121 metabolites quantified in both kits were available for further analyses, including 13 AAs, 14 ACs, 80 PCs (34 diacyl-, 26 acyl-alkyl, 10 lyso-PCs), 13 SM and the sum of hexoses. Abbreviations and full biochemical names of these metabolites are provided in Table 1.

Table 1 Abbreviations and full biochemical names of metabolites available in both Biocrates Absolute*IDQ*™ kits p150 and p180

Abbreviation	Full biochemical name	Abbreviation	Full biochemical name
Arg	Arginine	PC ae C30:0	Phosphatidylcholine acyl-alkyl C30:0
Gln	Glutamine	PC ae C30:2	Phosphatidylcholine acyl-alkyl C30:2
Gly	Glycine	PC ae C32:1	Phosphatidylcholine acyl-alkyl C32:1
His	Histidine	PC ae C32:2	Phosphatidylcholine acyl-alkyl C32:2
Met	Methionine	PC ae C34:0	Phosphatidylcholine acyl-alkyl C34:0
Orn	Ornithine	PC ae C34:1	Phosphatidylcholine acyl-alkyl C34:1
Phe	Phenylalanine	PC ae C34:2	Phosphatidylcholine acyl-alkyl C34:2
Pro	Proline	PC ae C34:3	Phosphatidylcholine acyl-alkyl C34:3
Ser	Serine	PC ae C36:0	Phosphatidylcholine acyl-alkyl C36:0
Thr	Threonine	PC ae C36:1	Phosphatidylcholine acyl-alkyl C36:1
Trp	Tryptophan	PC ae C36:2	Phosphatidylcholine acyl-alkyl C36:2
Tyr	Tyrosine	PC ae C36:3	Phosphatidylcholine acyl-alkyl C36:3
Val	Valine	PC ae C36:4	Phosphatidylcholine acyl-alkyl C36:4
H1	Hexose	PC ae C36:5	Phosphatidylcholine acyl-alkyl C36:5
C0	Carnitine	PC ae C38:0	Phosphatidylcholine acyl-alkyl C38:0
C2	Acetylcarnitine	PC ae C38:1	Phosphatidylcholine acyl-alkyl C38:1
C3	Propionylcarnitine	PC ae C38:2	Phosphatidylcholine acyl-alkyl C38:2
C5-OH (C3-DC-M)	Hydroxyvalerylcarnitine-(Methylmalonylcarnitine)	PC ae C38:3	Phosphatidylcholine acyl-alkyl C38:3
C7-DC	Pimelylcarnitine	PC ae C38:4	Phosphatidylcholine acyl-alkyl C38:4
C9	Nonaylcarnitine	PC ae C38:5	Phosphatidylcholine acyl-alkyl C38:5
C10	Decanoylcarnitine	PC ae C38:6	Phosphatidylcholine acyl-alkyl C38:6
C10:2	Decadienylcarnitine	PC ae C40:1	Phosphatidylcholine acyl-alkyl C40:1
C14:1	Tetradecenoylcarnitine	PC ae C40:2	Phosphatidylcholine acyl-alkyl C40:2
C14:2	Tetradecadienylcarnitine	PC ae C40:3	Phosphatidylcholine acyl-alkyl C40:3
C16	Hexadecanoylcarnitine	PC ae C40:4	Phosphatidylcholine acyl-alkyl C40:4
C18	Octadecanoylcarnitine	PC ae C40:5	Phosphatidylcholine acyl-alkyl C40:5
C18:1	Octadecenoylcarnitine	PC ae C40:6	Phosphatidylcholine acyl-alkyl C40:6
C18:2	Octadecadienylcarnitine	PC ae C42:1	Phosphatidylcholine acyl-alkyl C42:1
PC aa C28:1	Phosphatidylcholine diacyl C28:1	PC ae C42:2	Phosphatidylcholine acyl-alkyl C42:2
PC aa C30:0	Phosphatidylcholine diacyl C30:0	PC ae C42:3	Phosphatidylcholine acyl-alkyl C42:3
PC aa C32:0	Phosphatidylcholine diacyl C32:0	PC ae C42:4	Phosphatidylcholine acyl-alkyl C42:4
PC aa C32:1	Phosphatidylcholine diacyl C32:1	PC ae C42:5	Phosphatidylcholine acyl-alkyl C42:5
PC aa C32:2	Phosphatidylcholine diacyl C32:2	PC ae C44:3	Phosphatidylcholine acyl-alkyl C44:3
PC aa C32:3	Phosphatidylcholine diacyl C32:3	PC ae C44:4	Phosphatidylcholine acyl-alkyl C44:4
PC aa C34:1	Phosphatidylcholine diacyl C34:1	PC ae C44:5	Phosphatidylcholine acyl-alkyl C44:5
PC aa C34:2	Phosphatidylcholine diacyl C34:2	PC ae C44:6	Phosphatidylcholine acyl-alkyl C44:6
PC aa C34:3	Phosphatidylcholine diacyl C34:3	lysoPC a C14:0	lysoPhosphatidylcholine alkyl C14:0
PC aa C34:4	Phosphatidylcholine diacyl C34:4	lysoPC a C16:0	lysoPhosphatidylcholine alkyl C16:0
PC aa C36:0	Phosphatidylcholine diacyl C36:0	lysoPC a C16:1	lysoPhosphatidylcholine alkyl C16:1
PC aa C36:1	Phosphatidylcholine diacyl C36:1	lysoPC a C17:0	lysoPhosphatidylcholine alkyl C17:0

Abbreviation	Full biochemical name	Abbreviation	Full biochemical name
PC aa C36:2	Phosphatidylcholine diacyl C36:2	lysoPC a C18:0	lysoPhosphatidylcholine alkyl C18:0
PC aa C36:3	Phosphatidylcholine diacyl C36:3	lysoPC a C18:1	lysoPhosphatidylcholine alkyl C18:1
PC aa C36:4	Phosphatidylcholine diacyl C36:4	lysoPC a C18:2	lysoPhosphatidylcholine alkyl C18:2
PC aa C36:5	Phosphatidylcholine diacyl C36:5	lysoPC a C20:3	lysoPhosphatidylcholine alkyl C20:3
PC aa C36:6	Phosphatidylcholine diacyl C36:5	lysoPC a C20:4	lysoPhosphatidylcholine alkyl C20:4
PC aa C38:0	Phosphatidylcholine diacyl C38:0	lysoPC a C28:1	lysoPhosphatidylcholine alkyl C28:1
PC aa C38:1	Phosphatidylcholine diacyl C38:1	SM C16:0	Sphingomyeline C16:0
PC aa C38:3	Phosphatidylcholine diacyl C38:3	SM C16:1	Sphingomyeline C16:1
PC aa C38:4	Phosphatidylcholine diacyl C38:4	SM C18:0	Sphingomyeline C18:0
PC aa C38:5	Phosphatidylcholine diacyl C38:5	SM C18:1	Sphingomyeline C18:1
PC aa C38:6	Phosphatidylcholine diacyl C38:6	SM C20:2	Sphingomyeline C20:2
PC aa C40:2	Phosphatidylcholine diacyl C40:2	SM C24:0	Sphingomyeline C24:0
PC aa C40:3	Phosphatidylcholine diacyl C40:3	SM C24:1	Sphingomyeline C24:1
PC aa C40:4	Phosphatidylcholine diacyl C40:4	SM C26:1	Sphingomyeline C26:1
PC aa C40:5	Phosphatidylcholine diacyl C40:5	SM (OH) C14:1	Hydroxysphingomyeline C14:1
PC aa C40:6	Phosphatidylcholine diacyl C40:6	SM (OH) C16:1	Hydroxysphingomyeline C16:1
PC aa C42:0	Phosphatidylcholine diacyl C42:0	SM (OH) C22:1	Hydroxysphingomyeline C22:1
PC aa C42:1	Phosphatidylcholine diacyl C42:1	SM (OH) C22:2	Hydroxysphingomyeline C22:2
PC aa C42:2	Phosphatidylcholine diacyl C42:2	SM (OH) C24:1	Hydroxysphingomyeline C24:1
PC aa C42:4	Phosphatidylcholine diacyl C42:4		
PC aa C42:5	Phosphatidylcholine diacyl C42:5		
PC aa C42:6	Phosphatidylcholine diacyl C42:6		

Floegel *et al.* [108] evaluated the reliability of metabolites measured with the Absolute*IDQ*™ p150 kit and computed the intraclass correlation coefficient (ICC) with regard to changes over a 4-months period in fasting individuals. The ICC is a measure of reproducibility and ranges between 0 and 1 with ICC=0 indicating no reproducibility and ICC=1 indicating perfect reproducibility (Equation 2). For the majority of measured metabolites, a fair to good reliability given by an ICC above 0.4 was reported (Figure 9) [109].

Equation 2 Interpretation of ICC (modified from Rosner (2011) [109])

ICC > 0.4	poor reproducibility
0.4 ≤ ICC < 0.75	fair to good reproducibility
ICC ≥ 0.75	excellent reproducibility

Therefore, those metabolites are reliable over time and it was assumed that comparable metabolic profiles would be present at the time of body weight gain within the follow-up period.

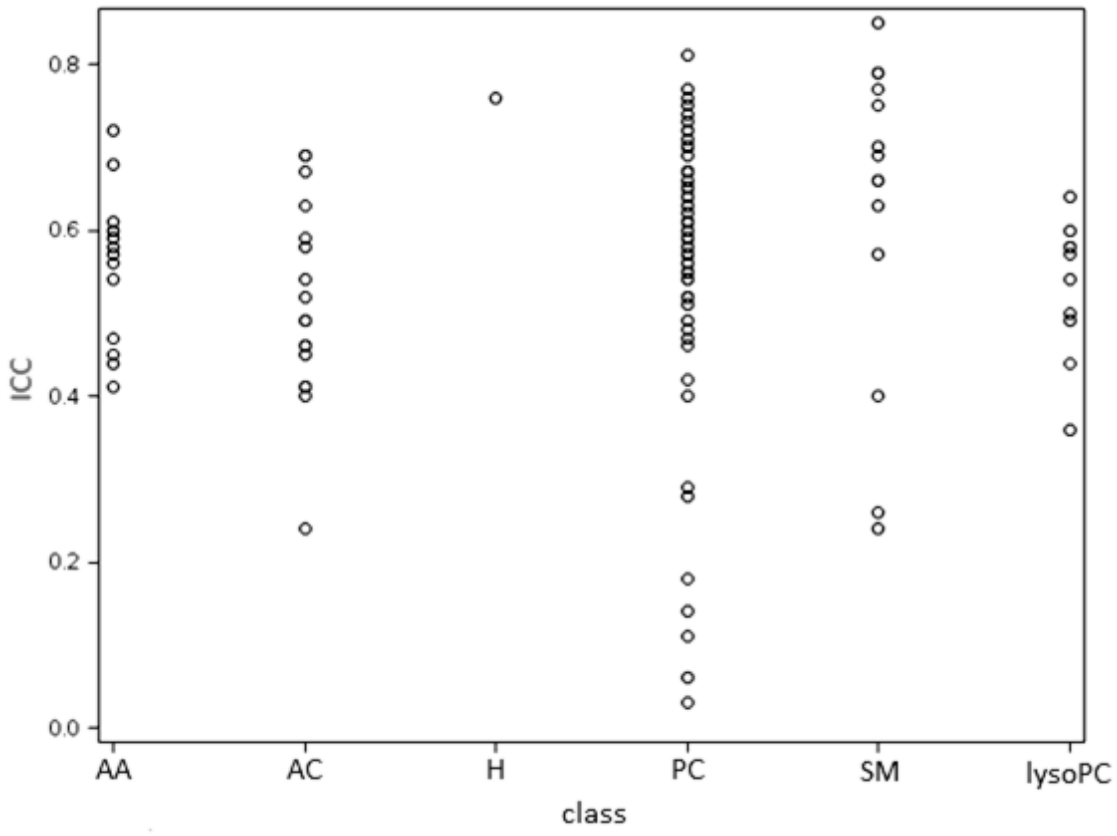

Figure 9 Intraclass correlation coefficients (ICCs) of 163 serum metabolites according to metabolite subclass measured over a 4-months period (modified according to Floegel *et al.* (2011) [108])

3.4.4. Statistical analysis

In order to increase comparability of results, metabolite concentrations were standardized to a mean of zero and a standard deviation of 1 for each sex separately.

Based on the observed distribution across all studies (objective 1), two different weight-gaining phenotypes could be defined through categorisation of average annual waist-hip difference in men and women: a waist-gaining (WG) and a hip-gaining (HG) phenotype. The lower 10% of the waist-hip difference were defined as the WG phenotype, whereas the upper 10% were defined as the HG phenotype.

Multiple logistic regression models were fitted with either the WG or the HG phenotype as the dependent and standardized metabolite concentrations as independent variables, adjusted for age at time of baseline examination, baseline BMI, baseline measures of waist and hip circumference, smoking status (never, former, current smoker) and prevalent and incident chronic diseases including myocardial infarction, stroke, any type of diabetes and cancer. Odds ratios (OR) were calculated between the phenotype of interest and the reference category.

Potential interactions were tested for the association between each metabolite and the covariate sex by including a multiplicative interaction term into the model. Because diacyl-PC C42:1, acyl-alkyl-PCs C44:3, C30:1, C32:1, C44:6 and SM C18:1 showed statistically significant interaction with sex, all logistic regression models were fitted sex-specific.

Because the logistic regression model was calculated for each metabolite, some of the results were expected to be false positive by chance due to type I error. Based on a common significance level of $\alpha=0.05$, there is a 5% chance of making a wrong decision. The number of false positive results would therefore increase due to multiple testing, e.g. m=121 tests with a significance level of $\alpha=0.05$ would result in 6 expected false positive results (Equation 3).

Equation 3 Expected false positive results without controlling for multiple testing

$$m * \alpha = \textit{expected overall false positive test results}$$

$$121 * 0.05 = 6.05$$

A possibility to address that point is to adjust the significance level α by setting a lower threshold. In general, multiple testing corrections successful decreases type I error and the number of false positives but on cost of increasing type II error and number of false

negative results. A very common but conservative approach is the use of the Bonferroni method [110]. This procedure controls the overall experiment-wise type I error rate through adjusting the threshold of the significance level by dividing this level by the number of all performed tests. This procedure would be appropriate if all tests were independent of each other. The analytical metabolomics dataset includes partly highly correlated metabolites (Appendix **2**), so that the assumption of independent tests is not appropriate. In case of highly correlated metabolites the number of type II error and therefore false negative results would increase even more when applying this method.

An alternative approach to control the problem of multiple testing is the use of the Benjamini-Hochberg method [111] which controls the False Discovery Rate (FDR). This approach is less conservative compared to Bonferroni and type II error appears less often [110]. Therefore the linear step-up FDR method was chosen using MULTTEST procedure of SAS 9.4 (SAS Institute Inc., Cary, NY). Based on the number of tests (m=121) performed in this analysis, this procedure works as described in Equation 4.

Equation 4 Benjamini-Hochberg FDR testing procedure (modified from Rosner (2011) [110])

(1) m = 121 separate tests with p-values $p_{(1)}\ldots p_{(121)}$ were conducted

(2) tests are renumbered (for convenience) and raw p-values are ordered according to $p_{(1)} \leq p_{(2)} \leq \ldots \leq p_{(n)} \leq \ldots \leq p_{(121)}$

(3) raw p-values are corrected using the equation $P_{FDR\,(n)} = \frac{m}{m-n}\ p_{(n)}$, e.g.

$P_{FDR\,(121)} = \frac{121}{121} p_{(121)}$

$P_{FDR\,(n)} = \frac{121}{n} p_{(n)}$

. .

. .

. .

$P_{FDR\,(1)} = \frac{121}{1} p_{(1)}$

(4) reject H_0 for the hypotheses with an FDR-p ≥ 0.05 and accept H_0 for remaining hypotheses

Several sensitivity analyses were performed to test the robustness of potential findings in a population without prevalent diseases at baseline or follow-up examination, a fasting population and for women in a population without menopause.

Subgroup analyses were performed for alcohol consumption (cut-off < 20 g/d for men and < 10 g/d for women), physical activity (cut-off < 1 h/week), age (cut-off ≤ 55 years) and abdominal obesity (waist circumference men ≥ 102 cm, women ≥ 88 cm).

Single study results of EPIC-Potsdam and KORA were then combined in a meta-analytical approach. The goal of a meta-analysis is to estimate a combined effect size through the computation of a weighted mean of the single study estimates [112].

In a fixed-effects model one true effect size is assumed which is present in all investigated studies and single estimates are distributed around this true effect with a variation that depends primarily on the sample size for each study. So the aim of a fixed-effects meta-analysis is to estimate this one true effect by the combination of weighted single study estimates [113]. Because distribution of the single study estimates around the true effect is largely determined by the within-study variance and large studies are in general more precise, each study is weighted by the inverse of its variance for the combination of study results [113].

In contrast, in a random-effects model many true effect sizes are assumed to be distributed across the investigated studies. So each study is estimating a different true effect size and the computed combined effect is a weighted mean of a distribution of true effects [114]. In this approach, the between-study variance is also taken into account for the combination of the single study results. So, the inverse of the total variance (sum of within- and between-study variance) is used for the combination of single study results with the goal of minimising both sources of variance [114].

For the present analysis it was assumed that both studies measure the same effect size with a variation that is due to the sample size for each study. Because analysis in both studies were functionally identical, a fixed-effects model was used to combine the single study estimates [115].

To separate between potential relevant and irrelevant metabolites and ensure that a combined association is not only due to a very strong effect in just one study by chance alone, the combined estimate had to fulfil two criteria to be regarded as true association: 1) single study estimates had to be consistent concerning effect direction and 2) the combined estimate had to be significant after multiple testing corrections.

All calculations were done using SAS release 9.4 (SAS Institute, Cary, NC, USA) in all studies. Fixed-effects meta-analyses were performed using the statistical software ‘R’,

version 3.1.2 [116] with the R package ‘meta’, version 4.1-0 [117]. To correct for multiple testing, the FDR was controlled at 0.05 using the Benjamini-Hochberg method [111].

3.5. Comparison of results from two approaches (objective 3)

3.5.1. Study population

This analysis is based on data from EPIC-Potsdam. The final analytical study population included 1,644 weight-gaining individuals, 1,015 women and 629 men. Detailed information on inclusion and exclusion criteria have been described in detail afore (chapter 3.2).

3.5.2. Statistical analysis

A principal component analysis (PCA) was conducted with all quality controlled metabolites of EPIC-Potsdam. The PCA as an unsupervised data reduction method is examining the variance of the data [118] and makes use of the fact that in general many metabolites remain almost constant between groups and explain only little variance. Thus, the metabolites that are often highly correlated and explain a large variation in the dataset are grouped to principal components [119]. Especially within subclasses, metabolites are often highly correlated. In chapter 3.4.4, this dependency was addressed by using the FDR-method as a less conservative approach of multiple testing correction instead of e.g. the Bonferroni method [111]. An alternative approach to address the highly correlated data is the use of a data reduction method such as PCA, which is also very common in the field of metabolomics [119]. Highly correlated metabolites are summarised and aggregated to a linear combination of the individual metabolites. The smaller numbers of obtained principal components explain in general a large amount of the between-person-variation in the underlying dataset, metabolites that do not differ between persons will not be considered in the interpretation of the principal components. Nevertheless, these metabolites are still part of the derived linear combination.

PCA was only conducted on basis of EPIC-Potsdam data. Because PCA requires each observed variable to be normally distributed, all metabolites where standardized to a mean of 0 and a standard deviation of 1. The number of principal components for further analysis was derived through a combination of criteria: remaining principal components should have at least an eigenvalue of 1 and the proportion of variance accounted for by the number of retained components should account for the major fraction of proportion of variance in the targeted metabolomics dataset (at least 50%). The proportion was calculated according to Equation 5.

Equation 5 Proportion of variance accounted for by principal components

$$\text{proportion of variance} = \frac{\text{proportion of the eigenvalue for the principal components}}{\text{total eigenvalues of the correlation matrix}}$$

To obtain uncorrelated sex-specific principal components, orthogonal varimax rotation method was performed with the required number of components using the FACTOR procedure of SAS 9.4 (SAS Institute Inc, Cary, NY) for men and women, separately. With the varimax-rotation the explained variance of the required principal components is redistributed on the rotated principal components. In general, this will lead to an increased interpretability of components [120]. For further interpretation and identification, relevant metabolites had to be uniquely assignable to only one principal component with a meaningful factor loading of 0.6.

To investigate the association of the obtained principal components with the WG and HG phenotypes, sex-specific multiple logistic regression models specified in chapter 3.4.4 were performed with the rotated principal components as independent variables. Because components are not 100% independent and several tests were performed, results were corrected for multiple testing using the FDR-method [111].

To investigate whether potential significant associations are caused by high loading metabolites of associated principal components or by metabolic constellations as a whole including as well low loading metabolites, simplified principal components were built. Therefore, only metabolites with a meaningful factor loading on the principal component of interest and their corresponding factor loading were used to form the corresponding simplified linear combinations for each principal component. The simplified principal components were again treated as independent variables in the multiple logistic regression models. Logistic regression results for original and simplified principal components were then compared regarding strength and direction of association.

Finally, results from the logistic regression approaches using either single metabolites or principal components as independent variables were compared with each other with regard to strength and direction of associations.

All statistical analyses were performed using statistical software SAS 9.4 (SAS Institute Inc, Cary, NY).

4. Results

4.1. Characteristics of the analytical study populations of EPIC-Potsdam, KORA and DEGS

Baseline characteristics and average annual changes of anthropometric measures of participants stratified by sex for each study are shown in Table 2.

The majority of weight gainers in the studies were categorised as overweight according to their BMI with women in EPIC-Potsdam having the lowest (25.1 ± 4.4 kg/m²) and women in KORA having the highest average baseline BMI (28.3 ± 4.6 kg/m²). In all studies, men had statistically significantly higher initial body weight, waist circumference and WHR and consumed more alcohol compared to women (all p<.0001). Average annual weight change was comparable between men and women in each study ranging from 0.52 ± 0.42 kg/yr for women in DEGS up to 0.63 ± 0.54 kg/yr for men in EPIC-Potsdam (all sex-differences not significant). Participants of KORA had the highest level of waist circumference (89.1 ± 10.9 cm for women, 100.1 ± 9.1 cm for men), highest percentage of abdominally obese people (51% for women, 35% for men) and suffered more often from chronic diseases compared to participants of EPIC-Potsdam and DEGS. EPIC-Potsdam participants had the lowest initial measures of body weight, waist and hip circumference but the highest rates of average annual changes in those body measures (0.63 ± 0.54 kg/yr, 1.12 ± 0.65 cm/yr waist and 0.53 ± 0.51 cm/yr hip changes for women; 0.62 ± 0.51 kg/yr, 1.21 ± 0.66 cm/yr waist and 0.48 ± 0.41 cm/yr hip changes for men). DEGS had the lowest rates of annual changes in waist circumference (0.52 ± 0.42 cm/yr for women; 0.55 ± 0.49 cm/yr for men) with men even showing a tendency of losing hip circumference on average (-0.11 ± 0.37 cm/yr).

Weight-gaining participants of DEGS had the lowest average age at baseline examination (43.3 ± 11.8 yr for women, 41.8 ± 12.8 yr for men, p=0.008) compared to EPIC-Potsdam (48.2 ± 9.0 yr for women, 51.2 ± 8.0 yr for men, p<.0001) and KORA with the highest average age (62.8 ± 5.2 yr for men, 62.6 ± 5.2 yr for men, p=0.69). Because of the high mean age at baseline examination, 94.7% of women in KORA were postmenopausal. In contrast, the analytical study populations of EPIC-Potsdam and DEGS included 22.8% and 39.8% postmenopausal women, respectively.

Lifestyle

There is a great gap regarding alcohol consumption between men and women with men having on average a three times higher alcohol consumption than women in all investigated cohorts (all p<.0001). In EPIC-Potsdam and KORA, men were more often current or former smokers than women. In the national survey DEGS a different distribution for smoking status could be observed showing that women were more often current or former smoker than men (all p<.0001).

The majority of participants were physically inactive with more than 50% of individuals in each cohort spending less than one hour per week with physical activities. In comparison, women were observed to have more moderate physical activity (1-2h/week) compared to men; in contrast men were slightly more active with regard to physical activity of more than 2 hours per week.

Phenotype characteristics

HG and WG phenotypes were defined as described in chapter 3.4.4. HG phenotype in both sexes was characterized by highest initial body weight (EPIC-Potsdam: 76.7 ± 16.4 kg for women, 78.8 ± 10.6 kg for men; KORA: 76.7 ± 11.3 kg for women, 89.9 ± 13.4 kg for men), largest initial waist and hip circumference (only exception hip circumference in men) but lowest rates of average annual body weight gain (EPIC-Potsdam: 0.49 ± 0.54 kg/yr for women, 0.41 ± 0.41 kg/yr for men; KORA: 0.71 ± 0.73 kg/yr for women; 0.46 ± 0.43 kg/yr for men). In both sexes, it could be observed that individuals with the HG phenotype had on average a higher WHR at baseline examination compared to the reference category or the WG phenotype. The WG phenotype was characterized by highest rates of weight gain and on average younger individuals compared to the HG phenotype. Besides, a tendency towards larger increase in waist than hip circumference in the reference categories was observed.

Table 2 Participant characteristics of weight-gaining individuals from EPIC-Potsdam, DEGS and KORA[a]

	DEGS[b]		EPIC-Potsdam		KORA	
Baseline characteristics	**Women (n=1,006)**	**Men (n=884)**	**Women (n=1,015)**	**Men (n=629)**	**Women (n=303)**	**Men (n=289)**
Age at recruitment (yr)	43.3 ± 11.8	41.8 ± 12.8	48.2 ± 9.0	51.5 ± 8.0	62.8 ± 5.2	62.6 ± 5.2
Body weight (kg)	68.3 ± 12.9	83.2 ± 11.8	66.9 ± 12.3	81.1 ± 11.4	71.9 ± 12.0	84.0 ± 11.2
Waist circumference (cm)	82.0 ± 11.4	94.9 ± 10.6	79.3 ± 11.4	93.0 ± 9.6	89.1 ± 10.9	100.1 ± 9.1
Hip circumference (cm)	104.0 ± 10.0	104.8 ± 6.3	100.4 ± 8.6	99.6 ± 6.0	106.9 ± 9.1	104.7 ± 6.6
BMI (kg/m²)	25.5 ± 4.7	26.6 ± 3.5	25.1 ± 4.4	26.5 ± 3.5	28.3 ± 4.6	28.2 ± 3.5
WHR	0.79 ± 0.06	0.90 ± 0.07	0.79 ± 0.07	0.93 ± 0.06	0.83 ± 0.06	0.96 ± 0.05
Prevalence of abdominal obesity (%)[c]	28.1	24.6	20.3	18.4	51.5	35.3
Alcohol consumption (g/d)	4.6 ± 8.4	15.5 ± 19.4	8.6 ± 10.5	24.5 ± 27.6	7.0 ± 10.7	25.9 ± 26.6
Average changes per year						
Weight (kg/yr)	0.52 ± 0.42	0.55 ± 0.49	0.63 ± 0.54	0.62 ± 0.51	0.56 ± 0.56	0.54 ± 0.49
Waist circumference (cm/yr)	0.64 ± 0.52	0.51 ± 0.52	1.12 ± 0.65	1.21 ± 0.66	0.86 ± 0.71	0.77 ± 0.62
Hip circumference (cm/yr)	0.17 ± 0.46	-0.11 ± 0.37	0.53 ± 0.51	0.48 ± 0.41	0.57 ± 0.59	0.44 ± 0.51
Smoking status						
Never smoker	286 (28.4)	308 (34.8)	598 (58.9)	181 (28.8)	214 (70.6)	91 (31.5)
Former smoker	166 (16.5)	234 (26.5)	237 (23.3)	287 (45.6)	67 (22.1)	147 (50.9)
Current smoker	554 (55.1)	342 (38.6)	180 (17.7)	161 (25.6)	22 (7.3)	51 (17.7)
Physical activity						
< 1 h/week	594 (59.1)	501 (56.8)	611 (60.1)	408 64.8	157 (51.8)	170 (58.8)
1-2h/week	209 (20.8)	150 (16.9)	263 (25.9)	110 17.5	94 (31.0)	66 (22.8)
> 2h/week	203 (20.1)	233 (26.3)	142 (14.0)	111 17.7	52 (17.2)	53 (18.3)
Prevalence of diseases	76 (7.6)	69 (7.8)	151 (14.9)	124 19.7	67 22.1	92 31.8

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio

[a] Values are mean ± SD or n (%)

[b] DEGS is standardized to the structure of the German population at 31.12.1997

[c] Abdominal obesity defined as waist circumference >88 cm (women) / >102 cm (men)

4.2. Tendencies to a specific body fat distribution in weight-gaining individuals (objective 1)

4.2.1. General tendencies

Based on the average annual waist-hip difference, it could be observed that two different types of weight gainers appeared. Weight-gaining individuals with the preferred deposition in the peripheral region with body weight gain rather increased their hip circumference than their waist circumference indicated by a negative value for waist-hip difference; individuals with the preferred deposition of body fat in the abdominal region with body weight gain rather increased their waist than their hip circumference indicated by a positive value for average annual waist-hip difference. Both weight gain types appeared in men as well as in women with the majority of individuals tend to increase more waist circumference. This clear tendency towards stronger increase of waist circumference compared to hip circumference could be observed in all studies independent of sex (Figure 10). Lowest waist-hip differences appeared within KORA (0.33 cm/yr for men; 0.29 cm/yr for women) whereas highest waist-hip differences appeared in EPIC-Potsdam (0.73 cm/yr for men; 0.60 cm/yr for women) (Table 3). Comparison of men and women in each study showed differences in EPIC-Potsdam and DEGS ($p<.0001$) but not in KORA ($p=0.41$). When comparing nationwide results of DEGS with regional results from EPIC-Potsdam and KORA, comparable tendencies across all studies could be observed.

Table 3 Average annual changes of waist-hip difference with corresponding 95% confidence intervals (CI) and their correlation with average annual weight change specific by study

	study	N	Waist-hip difference (cm/yr) Mean	(95% CI)	Pearson's r with weight change
Men	DEGS [a]	884	0.62	(0.59 ; 0.65)	0.27
	EPIC-Potsdam	629	0.73	(0.68 ; 0.78)	0.41
	KORA	289	0.33	(0.26 ; 0.40)	0.29
Women	DEGS [a]	1006	0.47	(0.43 ; 0.50)	0.13
	EPIC-Potsdam	1015	0.60	(0.56 ; 0.63)	0.10
	KORA	303	0.29	(0.22 ; 0.36)	0.005

[a] standardized to demographic structure of the German population at 31.12.1997

Potential bias due to drop-out was addressed in a sensitivity analysis. Inverse probability weights for reparticipation at follow-up were applied in EPIC-Potsdam with a high

reparticipation rate of 92% and in DEGS with a comparably low reparticipation rate of 47% [3]. Average annual waist-hip difference as the variable of interest was robust, 10^{th} and 90^{th} percentiles as cut-offs for the formation of WG and HG phenotype did not change in none of the studies (Appendix 1). Therefore no weighting for drop-out was performed in this analysis.

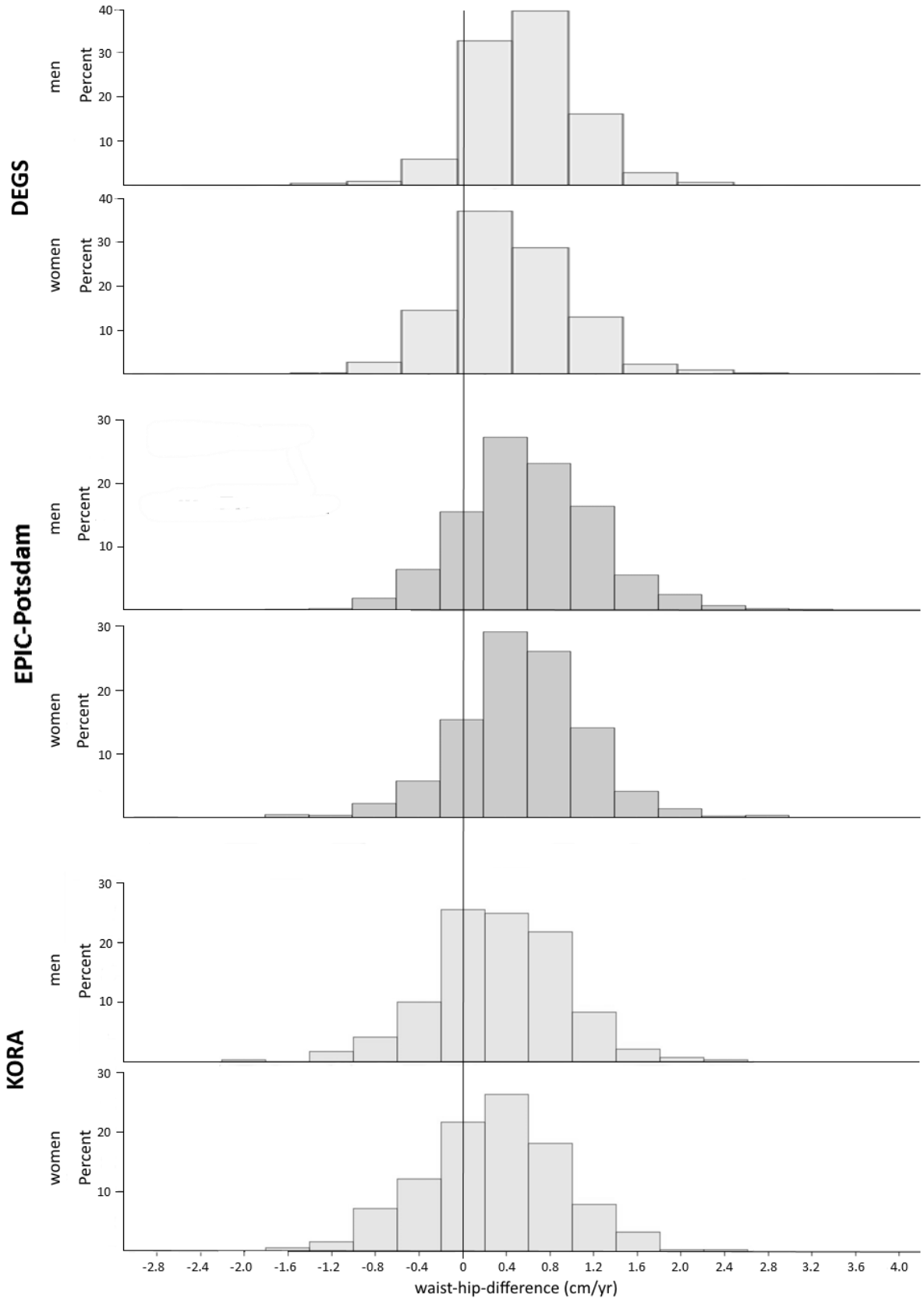

Figure 10 Distribution of average annual waist-hip difference in men and women stratified by study

4.2.2. Waist-hip difference in association to established anthropometric markers

Weight-gaining men showed a consistently stronger correlation between waist-hip difference and average annual weight change than women. Among men, EPIC-Potsdam showed the strongest correlation with r=0.41 (p<.0001) and DEGS the weakest correlation (r=0.27, p<.0001) (Figure 11); among women, DEGS showed the highest correlation (r=0.13, p<.0001) whereas KORA showed no statistically significant correlation between waist-hip difference and amount of body weight gain (r=0.005, p=0.9270) (Table 3 and Table 4). Thus, with a higher rate of average annual weight gain men tend to accumulate more body fat on their waists whereas women tend to accumulate body fat equally with increasing rates of average annual weight gain.

Table 4 Pearson correlation coefficients of waist-hip difference and established anthropometric markers by sex in EPIC-Potsdam

	Men (N = 629)		**Women (N = 1,015)**	
Average annual changes	**Pearson´s r**	**P**	**Pearson´s r**	**P**
Body weight (kg/yr)	0.41	<.0001	0.10	<.0001
BMI (kg/m²/yr)	0.39	<.0001	0.10	0.0015
Waist circumference (cm/yr)	0.80	<.0001	0.67	<.0001
Hip circumference (cm/yr)	-0.22	<.0001	-0.30	<.0001
WHR (U/yr)	0.99	<.0001	0.98	<.0001

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio

Exclusively in EPIC-Potsdam, additional established anthropometric markers were investigated regarding their association to the average annual waist-hip difference. It could be observed, that different correlations with this difference appeared for men and women (Table 4). Average annual changes of BMI and waist circumference were observed to be stronger positively correlated in men than in women; on the opposite women showed a stronger inverse correlation with average annual waist-hip difference than men.

It could be observed that results of Pearson correlation analyses reveal a nearly perfect correlation between average annual waist-hip difference and average annual changes in WHR (r=0.99 for men, r=0.98 for women, p<.0001). Thus, the majority of variance of average annual waist-hip difference could be as well explained by the average annual change in WHR.

Average annual changes of waist circumference showed stronger correlation with waist-hip difference than average changes of hip circumference in both men and women (Figure 11).

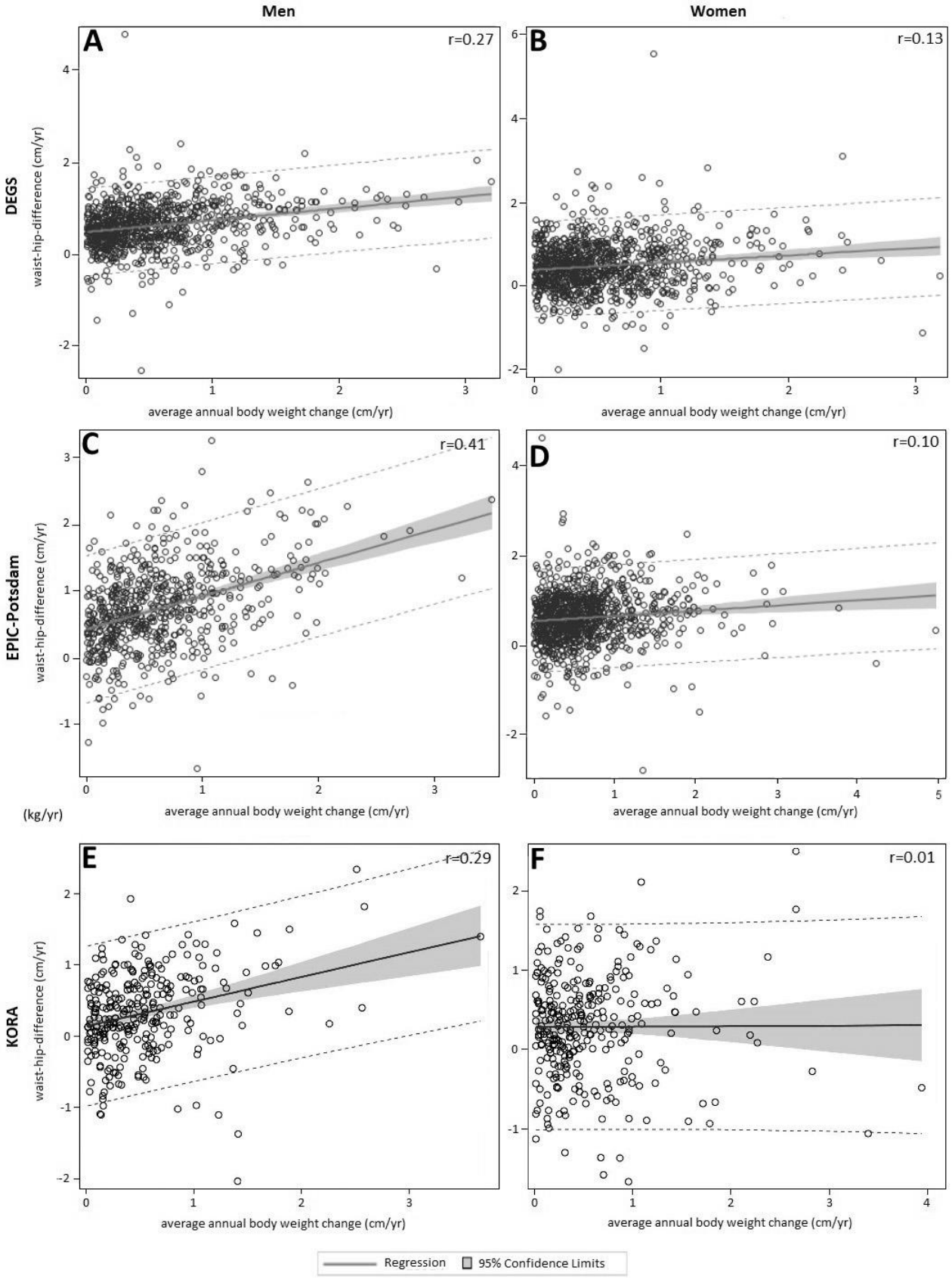

Figure 11 Scatter Plots with linear regression line and corresponding 95% confidence limits by sex and study

4.3. Identification of phenotype associated metabolites (objective 2)

Based on the observed distribution across all studies, two different weight-gaining phenotypes could be defined through categorisation of average annual waist-hip difference in men and women: a WG and a HG phenotype.

The lower 10% of the waist-hip difference defined as the HG phenotype was characterized by a significant tendency to increase more hip circumference in comparison to waist circumference in all studies for both men and women (Table 5). For men, mean waist-hip differences in the HG phenotype ranged from -0.22 cm/yr (95% Confidence Interval (CI): -0.29 cm/yr; -0.16 cm/yr) for DEGS to -0.77 cm/yr (95% CI: -0.90 cm/yr; -0.65 cm/yr) in KORA. For women, mean difference ranged from -0.45 cm/yr (95% CI: -0.52 cm/yr; -0.37 cm/yr) in EPIC-Potsdam to -0.90 cm/yr (95% CI: -1.00 cm/yr; -0.80 cm/yr) in KORA.

The upper 10% of the waist-hip difference defined as the WG phenotype was characterized by a significant tendency to increase more waist circumference in comparison to hip circumference. For men, mean waist-hip difference ranged from 1.32 cm/yr (95% CI: 1.21 cm/yr; 1.43 cm/yr) in KORA to 1.88 cm/yr (95% CI: 1.79 cm/yr; 1.97 cm/yr) in EPIC-Potsdam.

Table 5 Waist-hip difference and corresponding 95% confidence interval (CI) for defined phenotypes and overall specific by study

		DEGS [a]		EPIC-Potsdam		KORA	
Men	**Phenotype**	**Mean**	**(95% CI)**	**Mean**	**(95% CI)**	**Mean**	**(95% CI)**
All weight gainers		0.62	(0.59 ; 0.65)	0.73	(0.68 ; 0.78)	0.33	(0.26 ; 0.40)
	WG	1.49	(1.43; 1.56)	1.88	(1.79; 1.97)	1.32	(1.21; 1.43)
	HG	-0.22	(-0.29;-0.16)	-0.30	(-0.38;-0.22)	-0.77	(-0.90; -0.65)
Women							
All weight gainers		0.47	(0.43 ; 0.50)	0.60	(0.56 ; 0.63)	0.29	(0.22 ; 0.36)
	WG	1.54	(1.46; 1.62)	1.65	(1.57; 1.74)	1.42	(1.32; 1.53)
	HG	-0.46	(-0.52;-0.40)	-0.45	(-0.52;-0.37)	-0.90	(-1.00;-0.80)

[a] standardized to demographic structure of the German population at 31.12.1997

For EPIC-Potsdam and KORA, these two phenotypes were considered as the endpoints of sex-specific multiple logistic regression models. Meta-analytical combination of single-study results from EPIC-Potsdam and KORA identified 21 metabolites to be independently inversely associated with the WG phenotype in women, namely one AA, and 20 glycerophospholipids (19 PCs and 1 lysoPC).

4.3.1. Main results of the meta-analytical approach

The aromatic AA tryptophan, diacyl-PCs C32:3, C36:0, C38:0, C38:1, C42:2, C42:5, acyl-alkyl-PCs C32:2, C34:0, C36:0, C36:1, C36:2, C38:0, C38:2, C40:1, C40:2, C40:5, C40:6, C42:2, C42:3 and lyso-PC C17:0 could be identified as being associated with the WG phenotype in women (Table 6). Each of these metabolites showed inverse associations with increasing concentration. All statistically significant associations are within a range of OR 0.66-0.73 (p_{FDR} 0.0181-0.0474) i.e. if metabolite concentration of selected metabolites increases per one SD the chance of belonging to the WG phenotype decreases between 27 and 34%. The lowest ORs were observed for acyl-alkyl-PCs C40:6 (OR: 0.66, 95% CI: 0.52-0.82, p_{FDR}=0.0181), C42:3 (OR: 0.67, 95% CI: 0.53-0.83, p_{FDR}=0.0181), C38:0 (OR: 0.68, 95% CI: 0.54-0.85, p_{FDR}=0.0196) and C42:2 (OR: 0.68, 95% CI: 0.54-0.84, p_{FDR}=0.0181). All associations of WG and HG phenotypes with baseline metabolite concentrations for each study and combined are reported in Table 6 to Table 9. After correction for multiple testing, associations with the WG phenotype in women remained significant; the combined and study-specific estimates of these associations are shown in Figure 13.

4.3.2. Women

4.3.2.1. Waist-gaining phenotype

Results obtained from the meta-analytical combination of single study estimates revealed 41 metabolites as being associated with the WG phenotype in women, 38 glycerophospholipids (15 diacyl-, 20 acyl-alkyl, three lyso-PCs), two ACs and one AA (Table 6).

With regard to the single study results, 32 metabolites in EPIC-Potsdam and 19 metabolites in KORA showed associations with the WG phenotype in women. The lowest ORs in EPIC-Potsdam were observed for acyl-alkyl-PCs C42:2 (OR: 0.62, 95% CI: 0.49-0.85, p=0.0017) and C40:6 (OR: 0.65, 95% CI: 0.50-0.84, p=0.0012) and diacyl-PC C42:2 (OR: 0.64, 95% CI: 0.49-0.85, p=0.0017). The lowest ORs for KORA were observed for diacyl-PC C28:1 (OR: 0.39, 95% CI: 0.23-0.67, p=0.0005), acyl-alkyl-PC C30:2 (OR: 0.45, 95% CI: 0.26-0.78, p=0.0049) and hydroxy-SM C14:1 (OR: 0.46, 95% CI: 0.27-0.79, p=0.0051). There was no metabolite showing a positive association to the HG phenotype, neither in the meta-analytical approach nor in the single study results.

In both studies, in particular PCs showed inverse associations. 28 out of 32 metabolites in EPIC-Potsdam and 14 out of 19 metabolites associated with the WG phenotype in KORA belonged to the subclass of PC showing consistent inverse associations with the WG phenotype. In addition, AAs in EPIC-Potsdam and SMs in KORA were as well inversely associated.

Taking multiple testing into account, aromatic AA tryptophan, diacyl-PCs C38:0, C42:2 and acyl-alkyl-PCs C40:6, C42:2 and C42:3 remained significant in their association in EPIC-Potsdam; no metabolite showed significant associations in KORA (Table 6).

4.3.2.2. Hip-gaining phenotype

Meta-analytical results revealed four metabolites as being associated with the HG phenotype in women, three glycerophospholipids (two diacyl- and one acyl-alkyl-PC) and one SM (Table 7).

With regard to single study results, four metabolites in EPIC-Potsdam and 16 metabolites in KORA showed associations with the HG phenotype in women. The lowest ORs for EPIC-Potsdam were observed for diacyl-PC C40:6 (OR: 0.71, 95% CI: 0.55-0.93, p=0.0125) and SM C24:0 (OR: 0.76, 95% CI: 0.59-0.98, p=0.0328); in contrast no metabolite showed inverse associations with the HG phenotype in KORA. In EPIC-Potsdam, highest

ORs were observed for ACs nonaylcarnitine (C9, OR: 1.31, 95% CI: 1.03-1.66, p=0.0304) and pimelylcarnitine (C7-DC, OR: 1.27, 95% CI: 1.00-1.61, p=0.0491), highest ORs in KORA were observed for acyl-alkyl-PCs C42:2 (OR: 2.10, 95% CI: 1.33-3.30, p=0.0014), C34:3 (OR: 2.09, 95% CI: 1.36-3.22, p=0.0008) and C32:2 (OR: 2.00, 95% CI: 1.32-3.03, p=0.0012) (Table 7).

Unexceptionally, all associated metabolites in KORA, which mainly belonged to the group of acyl-alkyl-PCs, showed positive associations with the HG phenotype.

Taking multiple testing into account none of the metabolites showed statistically significant associations with the HG phenotype in women, neither in the single studies nor in the meta-analytical approach.

For the 21 metabolites with a statistically significant association to the WG phenotype in the meta-analytical approach in women, the associations for both endpoints were compared to investigate if directions of observed associations for the two phenotypes differ. When comparing the results of both endpoints of metabolites showing statistically significant associations with the WG phenotype in women, it could be observed that 17 out of 21 showed contrasting non-significant effect directions for the HG phenotype (Figure 13). Only aromatic AA tryptophan, diacyl-PC C42:5 and acyl-alkyl-PCs C40:1 and C42:2 had the same effect direction but showed much weaker strength for association with the HG phenotype.

4.3.3. Men

4.3.3.1. Waist-gaining phenotype

Meta-analytical results revealed eight metabolites as being associated with the WG phenotype in men, five glycerophospholipids (three diacyl-, two acyl-alkyl-, one lyso-PC), one AC and two SMs (Table 8).

With regard to the single study results for men, 21 metabolites in EPIC-Potsdam and eight metabolites in KORA showed associations with the WG phenotype in men. The lowest ORs for EPIC-Potsdam were observed for diacyl-PCs C42:1 (OR: 0.56, 95% CI: 0.39-0.81, p=0.0017), C44:3 (OR: 0.60, 95% CI: 0.42-0.87, p=0.0065) and C32:1 (OR: 0.61, 95% CI: 0.42-0.88, p=0.0087); in contrast no metabolite showed inverse associations in KORA. In EPIC-Potsdam, SMs C18:0 (OR: 1.37, 95% CI: 1.01-1.86, p=0.0448) and C18:1 (OR: 1.36, 95% CI: 1.02-1.81, p=0.0352) showed positive associations with the WG phenotype in men;

in contrast SM C16:1 (OR: 1,77, 95% CI: 1.08-2.92, p=0.0244), AC hexadecanoylcarnitine (C16, OR: 1.71, 95% CI: 1.15-2,54, p=0.0079) and diacyl-PC C34:2 (OR: 1.69, 95% CI: 1.14-2.51, p=0.0096) showed the strongest positive associations in KORA.

In both studies, in particular PCs were associated with the WG phenotype in men. 19 out of 21 metabolites in EPIC-Potsdam and three out of eight metabolites in KORA were part of this subclass of phospholipids. PCs in EPIC-Potsdam showed consistent inverse associations whereas PCs in KORA showed positive associations. Furthermore, SMs were observed to be positively associated in both studies. Additionally, majority of associations in KORA were positive with seven out of eight metabolites being positively associated (Table 8).

None of the associations between metabolites and the WG phenotype remained statistically significant when taking multiple testing into account, neither in the meta-analytical approach nor in the single study results.

4.3.3.2. Hip-gaining phenotype

Meta-analytical results revealed four metabolites as being associated with the HG phenotype in men, one diacyl-PC, one AA and two ACs (Table 9)

With regard to single study results, four metabolites in EPIC-Potsdam and three metabolites in KORA were observed as being associated with the HG phenotype in men. An inverse association was observed for diacyl-PC C38:1 (OR: 0.67, 95% CI: 0.47-0.97, p=0.0326) for EPIC-Potsdam and for AAs glycine (OR: 0.47, 95% CI: 0.24-0.89, p=0.0217), phenylalanine (OR: 0.57, 95% CI: 0.34-0.96, p=0.0331) and valine (OR: 0.59, 95% CI: 0.36-0.98, p=0.0414). Positive associations were observed in EPIC-Potsdam for ACs octadecanoylcarnitine (C18:0, OR: 1.50, 95% CI: 1.12-2.00, p=0.0063), octadecenoylcarnitine (C18:1, OR: 1.40, 95% CI: 1.06-1.85, p=0.0167) and diacyl-PC C28:1 (OR: 1.35, 95% CI: 1.01-1.78, p=0.0395); in contrast no positive association could be observed for HG phenotype for men in KORA (Table 9).

4.3.4. Sensitivity and subgroup analyses

To test whether associations of identified metabolites are robust, two sensitivity analyses were performed in a subsample of individuals without any prevalent disease at baseline or follow-up examination and in a subsample of non-menopausal women.

In EPIC-Potsdam and KORA, sensitivity analyses showed that associations were independent of disease status and also appeared in the population without prevalent diseases (Appendix 3). Results of the subgroup analyses (without correcting for multiple testing) were consistent regarding strength and direction of the association compared to the overall analytical study population. All other results were not significant, whereas the majority of remaining results were as well consistent.

Because women in KORA had a comparably high mean age and the majority of women were postmenopausal, sensitivity analysis with regard to menopausal status was exclusively performed in EPIC-Potsdam. Results within this sub-population showed consistent results compared to the overall analytical study population indicating that the identified association is independent of menopausal status (Appendix 3).

Furthermore, several subgroup analyses were performed to investigate the robustness of the identified associations. Significant associations (without correction for multiple testing) in the subgroups of physical activity (cut-off <1h/week), age (cut-off >55 years), abdominal obesity (cut-offs waist circumference ≥102cm for men and ≥88cm for women) and alcohol consumption (cut-offs <20g/d for men and <10g/d for women) showed consistent results concerning strength and direction of associations. For the remaining non-significant associations in the investigated subgroups, the majority showed consistent strength and direction of associations (Appendix 4).

Table 6 Combined fixed-effects meta-analytic and study-specific association of metabolites with the waist-gaining phenotype in women [a]

	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p-value	meta weight	OR	LCL	UCL	uncorrected p-value	meta weight
Amino Acids														
Arginine	0.81	0.66	1.00	0.1428	0.81	0.64	1.04	0.0949	75.3%	0.80	0.53	1.22	0.3026	24.7%
Glutamine	0.87	0.71	1.07	0.3315	0.81	0.64	1.02	0.0686	76.5%	1.11	0.73	1.70	0.6169	23.5%
Glycine	0.89	0.73	1.09	0.3993	0.78	0.61	1.00	0.0453	66.6%	1.17	0.83	1.66	0.3650	33.4%
Histidine	0.94	0.77	1.15	0.6657	0.92	0.72	1.16	0.4815	73.8%	1.00	0.67	1.50	0.9834	26.2%
Methionine	0.81	0.64	1.03	0.2272	0.70	0.52	0.93	0.0143	69.4%	1.14	0.74	1.77	0.5544	30.6%
Ornithine	0.94	0.76	1.14	0.6395	0.88	0.69	1.11	0.2773	71.7%	1.11	0.76	1.62	0.6060	28.3%
Phenylalanine	0.82	0.66	1.02	0.2013	0.82	0.64	1.05	0.1141	75.8%	0.83	0.53	1.29	0.4084	24.2%
Proline	1.00	0.81	1.23	0.9995	1.00	0.80	1.26	0.9943	82.6%	0.99	0.60	1.62	0.9693	17.4%
Serine	0.88	0.71	1.08	0.3489	0.75	0.59	0.97	0.0279	68.6%	1.22	0.84	1.77	0.3053	31.4%
Threonine	0.95	0.76	1.18	0.7224	0.83	0.65	1.07	0.1498	76.5%	1.43	0.92	2.24	0.1150	23.5%
Tryptophan	0.71	0.57	0.88	0.0253*	0.67	0.52	0.85	0.0013	75.8%	0.88	0.56	1.36	0.5580	24.2%
Tyrosine	0.93	0.75	1.15	0.6229	0.88	0.69	1.13	0.3076	75.3%	1.10	0.71	1.69	0.6725	24.7%
Valine	0.91	0.71	1.15	0.5541	0.83	0.62	1.10	0.1947	69.7%	1.12	0.73	1.74	0.5991	30.3%
Hexose														
H1	1.00	0.79	1.26	0.9995	0.92	0.70	1.21	0.5631	70.4%	1.21	0.79	1.85	0.3759	29.6%
Acylcarnitines														
C0	1.02	0.83	1.25	0.9018	0.97	0.77	1.22	0.7931	75.8%	1.20	0.80	1.81	0.3826	24.2%
C2	0.92	0.75	1.13	0.5541	0.89	0.71	1.12	0.3196	80.6%	1.05	0.66	1.67	0.8461	19.4%
C3	1.06	0.87	1.30	0.6657	1.01	0.80	1.28	0.9401	74.9%	1.25	0.83	1.87	0.2852	25.1%
C5-OH (C3-DC-M)	1.14	0.94	1.38	0.3315	1.16	0.94	1.43	0.1724	80.0%	1.07	0.70	1.64	0.7450	20.0%
C7-DC	0.88	0.71	1.09	0.3853	0.92	0.73	1.16	0.4776	86.7%	0.66	0.37	1.19	0.1714	13.3%
C9	0.90	0.73	1.12	0.4898	0.94	0.75	1.19	0.6249	83.4%	0.72	0.43	1.21	0.2148	16.6%
C10	0.81	0.63	1.05	0.2755	0.85	0.64	1.13	0.2646	82.4%	0.65	0.35	1.21	0.1749	17.6%
C10:2	1.04	0.85	1.27	0.7788	1.07	0.86	1.34	0.5337	78.3%	0.93	0.61	1.42	0.7330	21.7%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p-value	meta weight	OR	LCL	UCL	uncorrected p-value	meta weight
C14:1	0.86	0.69	1.07	0.3315	0.88	0.70	1.12	0.2972	81.5%	0.77	0.47	1.28	0.3165	18.5%
C14:2	0.93	0.76	1.14	0.6039	0.92	0.73	1.15	0.4503	79.2%	0.98	0.63	1.52	0.9386	20.8%
C16	0.77	0.62	0.96	0.0815	0.82	0.64	1.04	0.1041	79.4%	0.63	0.39	1.02	0.0613	20.6%
C18	0.79	0.63	0.98	0.1102	0.82	0.64	1.05	0.1175	79.7%	0.67	0.41	1.10	0.1137	20.3%
C18:1	0.85	0.69	1.05	0.2965	0.86	0.68	1.09	0.2191	79.9%	0.81	0.50	1.31	0.3902	20.1%
C18:2	0.98	0.80	1.19	0.8721	0.95	0.76	1.19	0.6415	81.2%	1.10	0.69	1.76	0.6883	18.8%
Diacyl-Phosphatidylcholines														
PC aa C28:1	0.79	0.63	0.98	0.1037	0.91	0.72	1.15	0.4268	83.0%	0.39	0.23	0.67	0.0005	17.0%
PC aa C30:0	0.88	0.71	1.08	0.3488	0.92	0.73	1.17	0.5041	77.6%	0.73	0.47	1.13	0.1563	22.4%
PC aa C32:0	0.84	0.68	1.05	0.2926	0.82	0.64	1.05	0.1184	77.1%	0.92	0.58	1.46	0.7313	22.9%
PC aa C32:1	0.90	0.72	1.12	0.4773	0.89	0.69	1.14	0.3598	78.1%	0.91	0.57	1.47	0.7124	21.9%
PC aa C32:2	0.85	0.69	1.06	0.2995	0.93	0.73	1.18	0.5345	79.5%	0.62	0.39	0.99	0.0456	20.5%
PC aa C32:3	0.69	0.55	0.86	0.0196*	0.69	0.54	0.88	0.0034	78.4%	0.69	0.43	1.11	0.1297	21.6%
PC aa C34:1	0.88	0.71	1.09	0.3901	0.93	0.73	1.17	0.5253	80.7%	0.73	0.45	1.18	0.1974	19.3%
PC aa C34:2	0.88	0.71	1.08	0.3594	0.94	0.75	1.18	0.5856	81.5%	0.66	0.41	1.07	0.0916	18.5%
PC aa C34:3	0.85	0.69	1.05	0.2965	0.85	0.67	1.08	0.1813	76.6%	0.87	0.56	1.33	0.5173	23.4%
PC aa C34:4	0.96	0.78	1.18	0.7788	1.05	0.83	1.31	0.6993	80.2%	0.68	0.43	1.07	0.0947	19.8%
PC aa C36:0	0.70	0.56	0.88	0.0229*	0.69	0.54	0.89	0.0043	76.5%	0.74	0.47	1.16	0.1894	23.5%
PC aa C36:1	0.87	0.71	1.07	0.3409	0.90	0.71	1.15	0.3995	75.9%	0.78	0.51	1.19	0.2417	24.1%
PC aa C36:2	0.87	0.71	1.07	0.3315	0.92	0.73	1.16	0.4729	79.1%	0.70	0.45	1.09	0.1174	20.9%
PC aa C36:3	0.91	0.74	1.12	0.5111	1.00	0.80	1.26	0.9989	83.2%	0.56	0.34	0.94	0.0279	16.8%
PC aa C36:4	1.03	0.84	1.27	0.8400	1.12	0.90	1.40	0.3177	84.6%	0.66	0.39	1.11	0.1172	15.4%
PC aa C36:5	0.85	0.66	1.08	0.3315	0.84	0.64	1.12	0.2315	76.1%	0.86	0.52	1.41	0.5475	23.9%
PC aa C36:6	0.77	0.61	0.97	0.0961	0.81	0.62	1.06	0.1206	76.0%	0.65	0.41	1.05	0.0767	24.0%
PC aa C38:0	0.69	0.56	0.86	0.0196*	0.67	0.52	0.86	0.0016	76.1%	0.78	0.50	1.22	0.2796	23.9%
PC aa C38:1	0.69	0.55	0.87	0.0229*	0.73	0.56	0.95	0.0173	76.0%	0.59	0.37	0.95	0.0281	24.0%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p-value	meta weight	OR	LCL	UCL	uncorrected p-value	meta weight
PC aa C38:3	0.94	0.75	1.18	0.7030	1.09	0.85	1.39	0.5081	82.4%	0.48	0.28	0.82	0.0068	17.6%
PC aa C38:4	1.00	0.81	1.23	0.9995	1.10	0.87	1.38	0.4191	81.6%	0.66	0.41	1.07	0.0900	18.4%
PC aa C38:5	0.85	0.68	1.05	0.2965	0.88	0.69	1.13	0.3184	78.5%	0.74	0.46	1.18	0.2025	21.5%
PC aa C38:6	0.76	0.61	0.94	0.0572	0.81	0.63	1.04	0.0986	77.0%	0.60	0.38	0.94	0.0259	23.0%
PC aa C40:2	0.75	0.59	0.97	0.0996	0.71	0.52	0.97	0.0321	66.1%	0.85	0.55	1.30	0.4479	33.9%
PC aa C40:3	0.76	0.60	0.95	0.0789	0.75	0.58	0.99	0.0389	72.8%	0.77	0.50	1.19	0.2393	27.2%
PC aa C40:4	0.98	0.79	1.21	0.9018	1.05	0.83	1.33	0.6710	82.2%	0.70	0.43	1.17	0.1749	17.8%
PC aa C40:5	0.85	0.68	1.07	0.3212	0.91	0.71	1.18	0.4835	77.5%	0.67	0.42	1.07	0.0960	22.5%
PC aa C40:6	0.74	0.59	0.93	0.0535	0.78	0.60	1.02	0.0669	74.3%	0.63	0.40	0.99	0.0465	25.7%
PC aa C42:0	0.79	0.64	0.98	0.1102	0.72	0.56	0.92	0.0097	73.4%	1.05	0.69	1.59	0.8310	26.6%
PC aa C42:1	0.77	0.62	0.96	0.0798	0.71	0.55	0.91	0.0070	73.5%	0.99	0.65	1.51	0.9569	26.5%
PC aa C42:2	0.72	0.56	0.91	0.0353*	0.64	0.49	0.85	0.0017	73.6%	0.97	0.61	1.54	0.9040	26.4%
PC aa C42:4	0.85	0.69	1.04	0.2646	0.84	0.67	1.06	0.1455	75.3%	0.86	0.57	1.30	0.4728	24.7%
PC aa C42:5	0.72	0.57	0.91	0.0353*	0.75	0.58	0.99	0.0393	76.1%	0.62	0.38	0.99	0.0464	23.9%
PC aa C42:6	0.76	0.61	0.95	0.0785	0.78	0.60	1.00	0.0540	76.6%	0.71	0.45	1.13	0.1474	23.4%
Acyl-alkyl-Phosphatidylcholines														
PC ae C30:0	0.76	0.61	0.94	0.0572	0.79	0.62	1.01	0.0560	78.2%	0.66	0.41	1.04	0.0723	21.8%
PC ae C30:2	0.74	0.59	0.94	0.0572	0.82	0.64	1.06	0.1306	83.2%	0.45	0.26	0.78	0.0049	16.8%
PC ae C32:1	0.80	0.65	0.98	0.1037	0.79	0.62	1.00	0.0483	74.9%	0.83	0.56	1.25	0.3827	25.1%
PC ae C32:2	0.73	0.59	0.90	0.0307*	0.72	0.57	0.92	0.0079	77.0%	0.74	0.48	1.15	0.1857	23.0%
PC ae C34:0	0.72	0.58	0.90	0.0353*	0.73	0.57	0.94	0.0150	75.9%	0.70	0.45	1.10	0.1201	24.1%
PC ae C34:1	0.79	0.64	0.98	0.0996	0.83	0.65	1.05	0.1208	77.8%	0.67	0.43	1.05	0.0797	22.2%
PC ae C34:2	0.92	0.75	1.13	0.5541	0.99	0.78	1.25	0.9054	77.8%	0.72	0.46	1.11	0.1403	22.2%
PC ae C34:3	0.86	0.70	1.05	0.2965	0.85	0.67	1.08	0.1764	74.5%	0.88	0.58	1.32	0.5259	25.5%
PC ae C36:0	0.72	0.58	0.91	0.0353*	0.71	0.55	0.91	0.0081	78.7%	0.79	0.48	1.29	0.3401	21.3%
PC ae C36:1	0.73	0.59	0.91	0.0353*	0.80	0.63	1.03	0.0781	79.7%	0.52	0.32	0.84	0.0082	20.3%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p-value	meta weight	OR	LCL	UCL	uncorrected p-value	meta weight
PC ae C36:2	0.71	0.56	0.88	0.0282*	0.77	0.60	0.99	0.0452	79.9%	0.49	0.30	0.82	0.0064	20.1%
PC ae C36:3	0.89	0.73	1.09	0.3993	0.95	0.75	1.19	0.6265	76.6%	0.74	0.49	1.12	0.1526	23.4%
PC ae C36:4	1.04	0.86	1.27	0.7619	1.06	0.85	1.32	0.6264	77.9%	1.00	0.66	1.52	0.9856	22.1%
PC ae C36:5	0.91	0.74	1.11	0.5029	0.90	0.72	1.13	0.3771	76.6%	0.94	0.62	1.42	0.7609	23.4%
PC ae C38:0	0.68	0.54	0.85	0.0196*	0.69	0.54	0.90	0.0053	76.5%	0.64	0.40	1.02	0.0596	23.5%
PC ae C38:1	0.94	0.78	1.15	0.6657	0.95	0.76	1.19	0.6287	75.4%	0.93	0.63	1.38	0.7321	24.6%
PC ae C38:2	0.75	0.60	0.93	0.0474*	0.77	0.61	0.99	0.0407	76.6%	0.67	0.43	1.05	0.0790	23.4%
PC ae C38:3	0.82	0.67	1.02	0.2013	0.94	0.74	1.19	0.6083	80.7%	0.48	0.29	0.77	0.0025	19.3%
PC ae C38:4	0.90	0.73	1.10	0.4563	0.95	0.76	1.20	0.6725	80.7%	0.71	0.44	1.13	0.1451	19.3%
PC ae C38:5	0.92	0.75	1.12	0.5248	0.89	0.71	1.12	0.3295	77.8%	1.00	0.65	1.53	0.9971	22.2%
PC ae C38:6	0.78	0.63	0.96	0.0798	0.78	0.61	0.99	0.0419	76.8%	0.77	0.49	1.20	0.2541	23.2%
PC ae C40:1	0.70	0.56	0.87	0.0196*	0.71	0.55	0.90	0.0058	77.2%	0.67	0.42	1.05	0.0807	22.8%
PC ae C40:2	0.73	0.58	0.91	0.0353*	0.78	0.60	1.00	0.0526	76.6%	0.59	0.37	0.93	0.0243	23.4%
PC ae C40:3	0.78	0.63	0.96	0.0815	0.83	0.65	1.05	0.1184	80.4%	0.61	0.38	0.98	0.0428	19.6%
PC ae C40:4	0.77	0.61	0.96	0.0792	0.78	0.61	1.00	0.0536	77.7%	0.72	0.45	1.14	0.1632	22.3%
PC ae C40:5	0.73	0.58	0.91	0.0353*	0.71	0.55	0.91	0.0079	76.8%	0.81	0.51	1.28	0.3605	23.2%
PC ae C40:6	0.66	0.52	0.82	0.0181*	0.65	0.50	0.84	0.0012	74.9%	0.67	0.43	1.05	0.0807	25.1%
PC ae C42:1	0.84	0.68	1.03	0.2510	0.84	0.67	1.07	0.1521	78.6%	0.83	0.53	1.30	0.4050	21.4%
PC ae C42:2	0.68	0.54	0.84	0.0181*	0.62	0.48	0.81	0.0004	70.2%	0.82	0.55	1.22	0.3276	29.8%
PC ae C42:3	0.67	0.53	0.83	0.0181*	0.67	0.52	0.86	0.0015	78.3%	0.67	0.41	1.07	0.0917	21.7%
PC ae C42:4	0.88	0.71	1.09	0.3898	0.89	0.69	1.14	0.3474	76.6%	0.86	0.55	1.34	0.5005	23.4%
PC ae C42:5	0.84	0.68	1.05	0.2926	0.81	0.63	1.04	0.0990	75.1%	0.96	0.62	1.48	0.8421	24.9%
PC ae C44:3	0.88	0.72	1.08	0.3669	0.90	0.72	1.13	0.3726	78.9%	0.82	0.53	1.27	0.3756	21.1%
PC ae C44:4	0.87	0.71	1.07	0.3315	0.85	0.67	1.07	0.1651	75.5%	0.94	0.62	1.43	0.7862	24.5%
PC ae C44:5	0.92	0.76	1.13	0.5726	0.90	0.72	1.14	0.3854	77.4%	1.00	0.65	1.53	0.9957	22.6%
PC ae C44:6	0.91	0.74	1.12	0.5248	0.85	0.67	1.07	0.1674	77.0%	1.18	0.76	1.81	0.4623	23.0%

Table continued on the next page

	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p-value	meta weight	OR	LCL	UCL	uncorrected p-value	meta weight
Lyso-Phosphatidylcholines														
lysoPC a C14:0	0.91	0.74	1.12	0.5248	0.94	0.74	1.19	0.6169	76.8%	0.82	0.53	1.26	0.3658	23.2%
lysoPC a C16:0	0.82	0.65	1.02	0.1931	0.77	0.60	0.99	0.0421	75.8%	0.98	0.62	1.52	0.9133	24.2%
lysoPC a C16:1	0.82	0.64	1.05	0.2729	0.77	0.58	1.02	0.0641	74.0%	1.00	0.62	1.60	0.9927	26.0%
lysoPC a C17:0	0.73	0.58	0.91	0.0353*	0.70	0.54	0.90	0.0062	74.3%	0.82	0.53	1.27	0.3767	25.7%
lysoPC a C18:0	0.81	0.65	0.99	0.1188	0.76	0.60	0.98	0.0302	72.1%	0.92	0.62	1.36	0.6848	27.9%
lysoPC a C18:1	0.79	0.63	0.99	0.1188	0.68	0.52	0.88	0.0037	75.9%	1.26	0.79	2.00	0.3334	24.1%
lysoPC a C18:2	0.87	0.70	1.08	0.3409	0.78	0.61	1.00	0.0476	74.8%	1.20	0.78	1.84	0.4040	25.2%
lysoPC a C20:3	0.87	0.70	1.08	0.3488	0.88	0.69	1.13	0.3071	79.5%	0.83	0.51	1.35	0.4462	20.5%
lysoPC a C20:4	0.92	0.74	1.14	0.5851	0.84	0.65	1.07	0.1506	77.5%	1.29	0.82	2.05	0.2681	22.5%
lysoPC a C28:1	0.87	0.71	1.08	0.3447	0.92	0.73	1.16	0.4767	80.9%	0.70	0.43	1.13	0.1405	19.1%
Sphingomyelins														
SM C16:0	1.00	0.82	1.22	0.9983	1.00	0.80	1.25	0.9942	80.0%	1.02	0.66	1.59	0.9267	20.0%
SM C16:1	0.98	0.80	1.21	0.9230	1.00	0.80	1.26	0.9806	81.1%	0.91	0.57	1.45	0.6823	18.9%
SM C18:0	1.03	0.84	1.27	0.8578	1.15	0.91	1.45	0.2386	81.7%	0.63	0.39	1.03	0.0643	18.3%
SM C18:1	0.95	0.77	1.17	0.7224	1.01	0.80	1.27	0.9677	80.6%	0.74	0.46	1.20	0.2249	19.4%
SM C20:2	0.85	0.69	1.06	0.2995	0.89	0.70	1.14	0.3654	77.6%	0.73	0.46	1.14	0.1647	22.4%
SM C24:0	1.03	0.84	1.25	0.8721	1.09	0.87	1.37	0.4428	76.8%	0.83	0.55	1.26	0.3853	23.2%
SM C24:1	1.01	0.83	1.23	0.9756	1.03	0.83	1.28	0.7874	79.9%	0.93	0.60	1.43	0.7278	20.1%
SM C26:1	1.04	0.85	1.28	0.7788	1.02	0.81	1.29	0.8413	77.2%	1.10	0.72	1.69	0.6515	22.8%
SM (OH) C14:1	0.85	0.68	1.05	0.2965	0.95	0.75	1.20	0.6590	84.3%	0.46	0.27	0.79	0.0051	15.7%
SM (OH) C16:1	0.87	0.70	1.07	0.3409	0.95	0.75	1.20	0.6363	84.1%	0.55	0.32	0.94	0.0285	15.9%
SM (OH) C22:1	0.91	0.74	1.11	0.4898	0.99	0.79	1.25	0.9303	80.8%	0.62	0.39	0.99	0.0474	19.2%
SM (OH) C22:2	0.87	0.71	1.08	0.3488	0.96	0.77	1.20	0.7201	85.1%	0.52	0.30	0.89	0.0171	14.9%
SM (OH) C24:1	0.99	0.81	1.21	0.9855	1.03	0.82	1.29	0.7889	78.2%	0.87	0.57	1.34	0.5286	21.8%

Abbreviations: a, acyl; ae, acyl-alkyl; aa, diacyl; PC, phosphatidylcholine; SM, sphingomyelin

[a] Adjusted for age, baseline measures of BMI, waist and hip circumference, smoking status, prevalent and incident diseases including myocardial infarction, stroke, diabetes and any type of cancer

*Statistically significant after correction for multiple testing (P_{FDR}<0.05)

Table 7 Combined fixed-effects meta-analytic and study-specific association of metabolites with the hip-gaining phenotype in women [a]

	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
Amino Acids														
Arginine	0.85	0.68	1.06	0.8165	0.91	0.71	1.16	0.4404	78.3%	0.66	0.41	1.06	0.0856	21.7%
Glutamine	1.07	0.87	1.32	0.8277	1.03	0.80	1.31	0.8368	73.9%	1.20	0.79	1.82	0.3862	26.1%
Glycine	1.06	0.86	1.31	0.8277	1.08	0.84	1.38	0.5589	72.4%	1.02	0.68	1.53	0.9257	27.6%
Histidine	1.01	0.82	1.25	0.9697	0.99	0.77	1.27	0.9132	71.6%	1.07	0.72	1.59	0.7405	28.4%
Methionine	1.00	0.81	1.23	0.9939	1.00	0.79	1.26	0.9870	76.0%	1.00	0.66	1.52	0.9885	24.0%
Ornithine	0.90	0.73	1.12	0.8165	0.93	0.72	1.19	0.5645	72.5%	0.84	0.56	1.26	0.3941	27.5%
Phenylalanine	0.92	0.75	1.14	0.8165	0.99	0.78	1.26	0.9507	76.2%	0.74	0.48	1.14	0.1679	23.8%
Proline	1.15	0.92	1.43	0.8165	1.20	0.93	1.53	0.1565	79.1%	0.99	0.61	1.60	0.9671	20.9%
Serine	1.14	0.92	1.41	0.8165	1.03	0.79	1.34	0.8318	67.0%	1.39	0.96	2.01	0.0852	33.0%
Threonine	1.03	0.82	1.29	0.9044	0.91	0.69	1.19	0.4843	69.7%	1.36	0.90	2.05	0.1430	30.3%
Tryptophan	0.97	0.79	1.20	0.8813	1.00	0.79	1.28	0.9831	76.4%	0.87	0.57	1.35	0.5415	23.6%
Tyrosine	1.05	0.86	1.29	0.8277	1.08	0.86	1.36	0.5094	76.1%	0.97	0.65	1.46	0.8979	23.9%
Valine	1.10	0.89	1.36	0.8165	1.18	0.93	1.50	0.1841	76.1%	0.89	0.58	1.37	0.6074	23.9%
Hexose														
H1	1.04	0.86	1.27	0.8277	1.06	0.85	1.31	0.6091	81.2%	0.99	0.64	1.55	0.9748	18.8%
Acylcarnitines														
C0	1.22	0.98	1.53	0.8165	1.18	0.91	1.53	0.2160	74.0%	1.35	0.87	2.09	0.1846	26.0%
C2	1.09	0.87	1.35	0.8165	1.08	0.83	1.39	0.5742	72.8%	1.11	0.73	1.69	0.6298	27.2%
C3	1.15	0.93	1.42	0.8165	1.12	0.88	1.44	0.3516	73.6%	1.21	0.80	1.83	0.3587	26.4%
C5-OH (C3-DC-M)	1.07	0.88	1.31	0.8277	1.12	0.89	1.41	0.3212	75.0%	0.93	0.62	1.38	0.7133	25.0%
C7-DC	1.16	0.93	1.44	0.8165	1.27	1.00	1.61	0.0491	83.3%	0.73	0.43	1.25	0.2557	16.7%
C9	1.24	1.00	1.54	0.8165	1.31	1.03	1.66	0.0304	79.9%	1.01	0.62	1.63	0.9717	20.1%
C10	1.18	0.99	1.40	0.8165	1.15	0.93	1.42	0.1868	69.9%	1.23	0.90	1.70	0.1941	30.1%
C10:2	1.01	0.83	1.24	0.9697	1.08	0.86	1.36	0.4967	80.5%	0.77	0.48	1.21	0.2572	19.5%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
C14:1	1.10	0.89	1.35	0.8165	1.09	0.85	1.40	0.5168	71.4%	1.12	0.75	1.66	0.5808	28.6%
C14:2	1.22	0.99	1.51	0.8165	1.26	0.99	1.61	0.0656	74.2%	1.12	0.74	1.70	0.5892	25.8%
C16	1.06	0.85	1.33	0.8277	1.08	0.84	1.41	0.5463	74.0%	1.00	0.65	1.55	0.9945	26.0%
C18	0.98	0.78	1.21	0.9044	0.92	0.71	1.19	0.5163	73.8%	1.16	0.76	1.77	0.5015	26.2%
C18:1	1.05	0.84	1.31	0.8277	1.00	0.77	1.30	0.9929	70.6%	1.19	0.79	1.79	0.4057	29.4%
C18:2	1.05	0.84	1.31	0.8277	0.98	0.76	1.26	0.8555	76.4%	1.33	0.84	2.11	0.2251	23.6%
Diacyl-Phosphatidylcholines														
PC aa C28:1	1.01	0.81	1.25	0.9832	0.97	0.75	1.26	0.8299	71.8%	1.10	0.73	1.66	0.6588	28.2%
PC aa C30:0	0.93	0.75	1.16	0.8277	0.94	0.73	1.21	0.6271	76.3%	0.91	0.58	1.43	0.6824	23.7%
PC aa C32:0	0.99	0.80	1.23	0.9697	0.91	0.71	1.17	0.4633	76.6%	1.30	0.83	2.03	0.2542	23.4%
PC aa C32:1	0.92	0.74	1.14	0.8165	0.93	0.73	1.19	0.5533	80.9%	0.88	0.53	1.45	0.6065	19.1%
PC aa C32:2	0.86	0.69	1.06	0.8165	0.90	0.70	1.16	0.4306	75.1%	0.73	0.47	1.12	0.1516	24.9%
PC aa C32:3	1.04	0.84	1.29	0.8344	0.95	0.74	1.22	0.7075	74.4%	1.33	0.87	2.03	0.1848	25.6%
PC aa C34:1	0.89	0.72	1.11	0.8165	0.86	0.67	1.10	0.2333	74.5%	1.01	0.66	1.55	0.9702	25.5%
PC aa C34:2	0.91	0.74	1.12	0.8165	0.85	0.67	1.09	0.1984	71.9%	1.07	0.72	1.58	0.7459	28.1%
PC aa C34:3	0.90	0.73	1.11	0.8165	0.89	0.69	1.13	0.3278	74.4%	0.95	0.63	1.44	0.8138	25.6%
PC aa C34:4	0.89	0.72	1.10	0.8165	0.95	0.74	1.22	0.6746	75.4%	0.73	0.47	1.12	0.1483	24.6%
PC aa C36:0	1.08	0.86	1.34	0.8277	0.93	0.72	1.19	0.5645	76.0%	1.71	1.10	2.67	0.0179	24.0%
PC aa C36:1	0.83	0.67	1.03	0.8165	0.82	0.64	1.05	0.1076	73.9%	0.88	0.58	1.33	0.5397	26.1%
PC aa C36:2	0.85	0.69	1.05	0.8165	0.80	0.62	1.03	0.0797	69.0%	0.97	0.67	1.41	0.8830	31.0%
PC aa C36:3	0.92	0.75	1.14	0.8165	0.92	0.72	1.17	0.4786	72.8%	0.95	0.64	1.41	0.7853	27.2%
PC aa C36:4	0.91	0.73	1.13	0.8165	0.90	0.70	1.15	0.3891	74.6%	0.95	0.62	1.46	0.8326	25.4%
PC aa C36:5	0.97	0.79	1.19	0.8813	0.90	0.71	1.14	0.3749	73.2%	1.21	0.82	1.79	0.3454	26.8%
PC aa C36:6	0.92	0.75	1.14	0.8165	0.88	0.69	1.12	0.3092	75.9%	1.07	0.70	1.63	0.7638	24.1%
PC aa C38:0	1.04	0.83	1.30	0.8344	0.86	0.66	1.12	0.2669	69.8%	1.62	1.08	2.44	0.0208	30.2%
PC aa C38:1	1.12	0.90	1.39	0.8165	1.06	0.83	1.36	0.6253	76.5%	1.33	0.85	2.07	0.2174	23.5%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
PC aa C38:3	0.84	0.68	1.05	0.8165	0.86	0.67	1.11	0.2569	73.5%	0.78	0.51	1.20	0.2569	26.5%
PC aa C38:4	0.86	0.70	1.07	0.8165	0.87	0.68	1.11	0.2665	75.0%	0.84	0.55	1.29	0.4281	25.0%
PC aa C38:5	0.94	0.76	1.16	0.8277	0.89	0.70	1.14	0.3535	75.4%	1.10	0.72	1.67	0.6713	24.6%
PC aa C38:6	0.88	0.71	1.10	0.8165	0.79	0.61	1.02	0.0678	73.9%	1.21	0.79	1.87	0.3740	26.1%
PC aa C40:2	1.04	0.84	1.28	0.8344	0.99	0.77	1.27	0.9340	74.1%	1.19	0.78	1.80	0.4165	25.9%
PC aa C40:3	1.06	0.84	1.33	0.8277	0.98	0.75	1.28	0.8874	72.0%	1.28	0.83	1.96	0.2661	28.0%
PC aa C40:4	0.86	0.70	1.06	0.8165	0.91	0.72	1.15	0.4097	80.5%	0.70	0.44	1.13	0.1480	19.5%
PC aa C40:5	0.79	0.64	0.98	0.8165	0.83	0.65	1.06	0.1326	78.0%	0.67	0.42	1.07	0.0962	22.0%
PC aa C40:6	0.79	0.63	0.99	0.8165	0.71	0.55	0.93	0.0125	70.8%	1.01	0.67	1.52	0.9767	29.2%
PC aa C42:0	1.11	0.88	1.39	0.8165	1.00	0.77	1.31	0.9773	71.9%	1.42	0.93	2.17	0.1043	28.1%
PC aa C42:1	1.05	0.84	1.32	0.8277	0.97	0.74	1.27	0.8170	72.8%	1.30	0.84	2.02	0.2336	27.2%
PC aa C42:2	1.09	0.89	1.34	0.8165	0.96	0.76	1.21	0.7429	79.8%	1.79	1.13	2.84	0.0136	20.2%
PC aa C42:4	0.91	0.73	1.14	0.8165	0.81	0.62	1.06	0.1173	68.6%	1.18	0.80	1.76	0.4013	31.4%
PC aa C42:5	0.92	0.74	1.15	0.8277	0.92	0.71	1.19	0.5068	72.8%	0.95	0.62	1.44	0.7946	27.2%
PC aa C42:6	0.95	0.77	1.17	0.8277	0.93	0.73	1.18	0.5522	75.0%	1.03	0.68	1.56	0.8945	25.0%
Acyl-alkyl-Phosphatidylcholines														
PC ae C30:0	1.16	0.93	1.46	0.8165	1.11	0.85	1.45	0.4527	73.5%	1.34	0.86	2.09	0.1983	26.5%
PC ae C30:2	1.06	0.84	1.33	0.8277	1.01	0.77	1.33	0.9441	72.3%	1.19	0.76	1.85	0.4423	27.7%
PC ae C32:1	1.14	0.91	1.42	0.8165	0.98	0.75	1.28	0.8736	70.1%	1.61	1.07	2.41	0.0211	29.9%
PC ae C32:2	1.25	1.00	1.56	0.8165	1.04	0.80	1.35	0.7749	71.5%	2.00	1.32	3.03	0.0012	28.5%
PC ae C34:0	1.11	0.89	1.39	0.8165	1.08	0.83	1.39	0.5778	74.6%	1.23	0.79	1.91	0.3521	25.4%
PC ae C34:1	1.19	0.95	1.49	0.8165	1.09	0.84	1.41	0.5432	72.9%	1.52	0.99	2.34	0.0539	27.1%
PC ae C34:2	1.13	0.91	1.42	0.8165	0.93	0.72	1.20	0.5708	75.6%	2.10	1.33	3.30	0.0014	24.4%
PC ae C34:3	1.14	0.91	1.44	0.8165	0.90	0.69	1.18	0.4482	71.6%	2.09	1.36	3.22	0.0008	28.4%
PC ae C36:0	1.03	0.83	1.27	0.8991	0.97	0.76	1.25	0.8177	75.3%	1.22	0.79	1.88	0.3751	24.7%
PC ae C36:1	1.05	0.84	1.31	0.8277	1.02	0.79	1.32	0.8952	74.2%	1.15	0.74	1.78	0.5282	25.8%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
PC ae C36:2	1.16	0.93	1.45	0.8165	1.07	0.83	1.39	0.5901	74.3%	1.46	0.94	2.26	0.0940	25.7%
PC ae C36:3	1.09	0.88	1.36	0.8165	0.90	0.70	1.17	0.4419	71.5%	1.75	1.16	2.64	0.0078	28.5%
PC ae C36:4	1.07	0.87	1.32	0.8277	1.00	0.78	1.28	0.9965	71.7%	1.27	0.86	1.89	0.2314	28.3%
PC ae C36:5	1.06	0.86	1.31	0.8277	0.95	0.74	1.21	0.6673	71.6%	1.41	0.95	2.09	0.0848	28.4%
PC ae C38:0	1.00	0.80	1.24	0.9923	0.89	0.69	1.14	0.3433	75.2%	1.43	0.92	2.21	0.1119	24.8%
PC ae C38:1	0.91	0.73	1.14	0.8165	0.90	0.69	1.16	0.4033	73.5%	0.96	0.62	1.47	0.8355	26.5%
PC ae C38:2	1.09	0.87	1.36	0.8165	1.10	0.85	1.42	0.4886	73.0%	1.07	0.70	1.64	0.7455	27.0%
PC ae C38:3	1.11	0.90	1.37	0.8165	1.11	0.88	1.41	0.3853	75.7%	1.10	0.72	1.68	0.6442	24.3%
PC ae C38:4	1.08	0.86	1.34	0.8277	0.99	0.77	1.28	0.9409	73.2%	1.35	0.88	2.06	0.1650	26.8%
PC ae C38:5	1.15	0.93	1.41	0.8165	1.01	0.79	1.29	0.9353	70.6%	1.56	1.06	2.29	0.0226	29.4%
PC ae C38:6	1.07	0.86	1.33	0.8277	0.93	0.72	1.19	0.5461	74.3%	1.62	1.06	2.47	0.0270	25.7%
PC ae C40:1	0.95	0.76	1.19	0.8277	0.87	0.67	1.12	0.2730	75.1%	1.26	0.81	1.97	0.3020	24.9%
PC ae C40:2	1.10	0.89	1.36	0.8165	1.00	0.78	1.28	0.9955	73.7%	1.43	0.94	2.15	0.0917	26.3%
PC ae C40:3	1.04	0.84	1.30	0.8344	0.93	0.72	1.21	0.5957	72.2%	1.40	0.92	2.13	0.1118	27.8%
PC ae C40:4	1.09	0.87	1.37	0.8165	1.01	0.77	1.32	0.9541	71.5%	1.32	0.87	2.02	0.1932	28.5%
PC ae C40:5	1.10	0.89	1.37	0.8165	0.99	0.77	1.27	0.9304	73.9%	1.50	0.99	2.29	0.0573	26.1%
PC ae C40:6	1.10	0.88	1.38	0.8165	0.92	0.71	1.20	0.5269	72.2%	1.75	1.14	2.68	0.0099	27.8%
PC ae C42:1	0.95	0.77	1.19	0.8277	0.94	0.73	1.21	0.6037	73.3%	1.01	0.66	1.54	0.9560	26.7%
PC ae C42:2	0.87	0.70	1.08	0.8165	0.80	0.62	1.03	0.0831	74.2%	1.09	0.72	1.67	0.6795	25.8%
PC ae C42:3	1.00	0.79	1.26	0.9939	0.85	0.65	1.11	0.2269	73.4%	1.57	1.00	2.47	0.0479	26.6%
PC ae C42:4	1.12	0.89	1.42	0.8165	0.99	0.76	1.31	0.9565	72.2%	1.55	1.00	2.42	0.0508	27.8%
PC ae C42:5	1.14	0.91	1.43	0.8165	1.04	0.80	1.36	0.7701	69.4%	1.41	0.94	2.10	0.0961	30.6%
PC ae C44:3	0.86	0.68	1.08	0.8165	0.84	0.64	1.10	0.2039	68.5%	0.91	0.61	1.37	0.6541	31.5%
PC ae C44:4	1.10	0.87	1.37	0.8165	1.06	0.81	1.39	0.6698	71.0%	1.19	0.78	1.81	0.4170	29.0%
PC ae C44:5	1.16	0.93	1.46	0.8165	1.07	0.82	1.41	0.6147	69.3%	1.39	0.92	2.09	0.1148	30.7%
PC ae C44:6	1.14	0.90	1.44	0.8165	1.01	0.77	1. 33	0.9264	72.3%	1.56	1.00	2.42	0.0492	27.7%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
Lyso-Phosphatidylcholines														
lysoPC a C14:0	0.94	0.76	1.17	0.8277	1.04	0.81	1.33	0.7703	77.8%	0.68	0.43	1.07	0.0969	22.2%
lysoPC a C16:0	0.89	0.71	1.11	0.8165	0.89	0.69	1.15	0.3756	75.1%	0.88	0.56	1.38	0.5708	24.9%
lysoPC a C16:1	0.96	0.80	1.16	0.8344	0.96	0.78	1.18	0.7150	83.1%	0.98	0.62	1.55	0.9280	16.9%
lysoPC a C17:0	1.11	0.89	1.39	0.8165	1.12	0.87	1.46	0.3801	74.4%	1.07	0.69	1.67	0.7585	25.6%
lysoPC a C18:0	0.83	0.67	1.03	0.8165	0.84	0.65	1.08	0.1683	73.2%	0.81	0.54	1.22	0.3131	26.8%
lysoPC a C18:1	0.95	0.78	1.17	0.8277	0.93	0.74	1.16	0.5046	82.5%	1.10	0.67	1.79	0.7034	17.5%
lysoPC a C18:2	0.96	0.76	1.21	0.8344	0.93	0.71	1.22	0.5951	74.1%	1.04	0.66	1.64	0.8589	25.9%
lysoPC a C20:3	0.90	0.73	1.11	0.8165	0.94	0.75	1.18	0.5991	82.7%	0.73	0.44	1.20	0.2146	17.3%
lysoPC a C20:4	0.94	0.76	1.17	0.8277	0.95	0.76	1.20	0.6899	86.4%	0.85	0.47	1.53	0.5856	13.6%
lysoPC a C28:1	0.99	0.78	1.25	0.9697	0.95	0.72	1.27	0.7421	67.7%	1.06	0.71	1.60	0.7703	32.3%
Sphingomyelins														
SM C16:0	1.05	0.84	1.32	0.8277	0.90	0.70	1.17	0.4400	73.8%	1.62	1.05	2.50	0.0307	26.2%
SM C16:1	1.16	0.92	1.46	0.8165	0.98	0.75	1.28	0.8785	72.2%	1.80	1.16	2.78	0.0084	27.8%
SM C18:0	0.94	0.76	1.17	0.8277	0.86	0.67	1.11	0.2436	73.2%	1.21	0.80	1.82	0.3753	26.8%
SM C18:1	1.06	0.85	1.32	0.8277	0.94	0.73	1.22	0.6328	71.6%	1.43	0.95	2.15	0.0901	28.4%
SM C20:2	1.22	0.98	1.51	0.8165	1.08	0.84	1.39	0.5493	72.7%	1.68	1.11	2.53	0.0138	27.3%
SM C24:0	0.79	0.64	0.98	0.8165	0.76	0.59	0.98	0.0338	72.1%	0.88	0.59	1.32	0.5388	27.9%
SM C24:1	1.01	0.81	1.25	0.9697	0.91	0.71	1.17	0.4667	71.1%	1.30	0.87	1.93	0.1980	28.9%
SM C26:1	0.96	0.77	1.19	0.8344	0.84	0.65	1.09	0.1832	72.0%	1.36	0.90	2.05	0.1468	28.0%
SM (OH) C14:1	1.18	0.94	1.47	0.8165	1.09	0.84	1.42	0.5300	71.5%	1.44	0.95	2.19	0.0860	28.5%
SM (OH) C16:1	1.14	0.92	1.41	0.8165	1.05	0.81	1.35	0.7283	73.0%	1.43	0.95	2.16	0.0893	27.0%
SM (OH) C22:1	0.89	0.72	1.11	0.8165	0.87	0.68	1.12	0.2901	74.1%	0.95	0.62	1.45	0.7975	25.9%
SM (OH) C22:2	1.07	0.85	1.34	0.8277	0.97	0.75	1.26	0.8162	74.5%	1.42	0.91	2.22	0.1222	25.5%
SM (OH) C24:1	1.01	0.82	1.25	0.9697	0.99	0.77	1.27	0.9260	72.9%	1.08	0.72	1.62	0.7032	27.1%

Abbreviations: a, acyl; ae, acyl-alkyl; aa, diacyl; PC, phosphatidylcholine; SM, sphingomyelin

[a] Adjusted for age, baseline measures of BMI, waist and hip circumference, smoking status, prevalent and incident diseases including myocardial infarction, stroke, diabetes and any type of cancer

Table 8 Combined fixed-effects meta-analytic and study-specific association of metabolites with the waist-gaining phenotype in men [a]

	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
Amino Acids														
Arginine	1.10	0.87	1.40	0.6891	0.94	0.69	1.29	0.7113	58.0%	1.37	0.95	1.97	0.0929	42.0%
Glutamine	1.10	0.87	1.39	0.6891	0.93	0.69	1.27	0.6662	58.8%	1.39	0.96	2.01	0.0792	41.2%
Glycine	0.91	0.68	1.22	0.7474	0.93	0.66	1.31	0.6791	72.8%	0.86	0.49	1.51	0.6030	27.2%
Histidine	1.10	0.86	1.41	0.7158	0.90	0.65	1.24	0.5127	58.8%	1.47	1.00	2.16	0.0509	41.2%
Methionine	1.03	0.80	1.31	0.8786	0.85	0.61	1.18	0.3274	55.1%	1.30	0.90	1.87	0.1671	44.9%
Ornithine	1.13	0.88	1.45	0.6891	0.85	0.61	1.19	0.3439	58.9%	1.69	1.14	2.50	0.0092	41.1%
Phenylalanine	1.12	0.88	1.43	0.6891	1.07	0.79	1.46	0.6627	60.8%	1.20	0.81	1.76	0.3664	39.2%
Proline	1.06	0.84	1.35	0.7849	1.01	0.75	1.38	0.9279	59.6%	1.14	0.79	1.66	0.4777	40.4%
Serine	0.91	0.70	1.19	0.7326	0.76	0.54	1.05	0.0986	63.1%	1.25	0.81	1.94	0.3111	36.9%
Threonine	0.79	0.60	1.04	0.6891	0.71	0.49	1.02	0.0649	55.8%	0.91	0.60	1.37	0.6538	44.2%
Tryptophan	1.06	0.83	1.36	0.7871	0.92	0.68	1.26	0.6143	64.1%	1.37	0.90	2.08	0.1386	35.9%
Tyrosine	1.07	0.84	1.36	0.7785	0.91	0.66	1.24	0.5444	57.7%	1.33	0.92	1.93	0.1254	42.3%
Valine	1.17	0.92	1.49	0.6891	1.01	0.74	1.37	0.9595	64.0%	1.52	1.02	2.28	0.0413	36.0%
Hexose														
H1	1.29	0.98	1.68	0.5684	1.33	0.97	1.84	0.0810	69.2%	1.19	0.73	1.92	0.4864	30.8%
Acylcarnitines														
C0	1.15	0.90	1.47	0.6891	1.06	0.79	1.43	0.7051	67.4%	1.36	0.89	2.09	0.1589	32.6%
C2	1.06	0.83	1.35	0.7871	0.96	0.69	1.33	0.8036	56.6%	1.20	0.83	1.74	0.3341	43.4%
C3	1.11	0.86	1.45	0.6891	1.02	0.73	1.42	0.9145	63.5%	1.30	0.84	2.01	0.2400	36.5%
C5-OH (C3-DC-M)	1.27	1.00	1.62	0.5556	1.27	0.94	1.73	0.1225	63.4%	1.27	0.85	1.90	0.2510	36.6%
C7-DC	1.13	0.89	1.42	0.6891	1.24	0.91	1.68	0.1708	60.1%	0.98	0.67	1.42	0.8971	39.9%
C9	1.02	0.79	1.31	0.9028	0.93	0.67	1.30	0.6702	57.0%	1.15	0.78	1.69	0.4718	43.0%
C10	1.07	0.84	1.34	0.7785	1.11	0.85	1.45	0.4630	74.6%	0.96	0.60	1.51	0.8452	25.4%
C10:2	1.06	0.83	1.34	0.7871	1.07	0.78	1.46	0.6882	58.1%	1.04	0.72	1.51	0.8163	41.9%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
C14:1	1.07	0.84	1.37	0.7781	1.01	0.73	1.39	0.9633	58.8%	1.17	0.80	1.72	0.4197	41.2%
C14:2	1.06	0.83	1.35	0.7871	0.99	0.72	1.36	0.9554	58.6%	1.15	0.79	1.69	0.4579	41.4%
C16	1.13	0.88	1.46	0.6891	0.86	0.62	1.19	0.3587	59.6%	1.71	1.15	2.54	0.0079	40.4%
C18	1.39	1.09	1.79	0.3628	1.26	0.92	1.72	0.1552	62.5%	1.66	1.10	2.48	0.0150	37.5%
C18:1	1.14	0.89	1.45	0.6891	0.96	0.70	1.31	0.8053	61.1%	1.47	1.00	2.17	0.0525	38.9%
C18:2	1.02	0.80	1.29	0.8989	0.86	0.63	1.18	0.3393	56.9%	1.28	0.89	1.84	0.1775	43.1%
Diacyl-Phosphatidylcholines														
PC aa C28:1	0.89	0.68	1.16	0.6891	0.84	0.60	1.17	0.2951	65.8%	0.99	0.62	1.56	0.9516	34.2%
PC aa C30:0	0.94	0.73	1.21	0.7871	0.84	0.60	1.18	0.3198	56.0%	1.07	0.73	1.58	0.7201	44.0%
PC aa C32:0	0.97	0.76	1.25	0.8617	0.81	0.57	1.15	0.2336	51.3%	1.18	0.82	1.68	0.3736	48.7%
PC aa C32:1	1.07	0.80	1.42	0.7871	0.71	0.42	1.19	0.1947	30.4%	1.27	0.90	1.80	0.1661	69.6%
PC aa C32:2	0.85	0.63	1.14	0.6891	0.80	0.56	1.15	0.2288	67.3%	0.95	0.57	1.60	0.8552	32.7%
PC aa C32:3	0.88	0.66	1.16	0.6891	0.70	0.50	0.98	0.0375	67.6%	1.40	0.86	2.29	0.1745	32.4%
PC aa C34:1	1.09	0.85	1.39	0.7326	0.85	0.61	1.19	0.3376	56.3%	1.49	1.02	2.18	0.0377	43.7%
PC aa C34:2	1.22	0.96	1.56	0.6891	1.01	0.74	1.37	0.9451	62.6%	1.69	1.14	2.51	0.0096	37.4%
PC aa C34:3	0.97	0.73	1.27	0.8617	0.69	0.47	1.01	0.0539	51.8%	1.40	0.94	2.08	0.0995	48.2%
PC aa C34:4	0.89	0.68	1.16	0.6891	0.79	0.57	1.10	0.1666	66.8%	1.12	0.70	1.78	0.6291	33.2%
PC aa C36:0	0.88	0.67	1.14	0.6891	0.72	0.51	1.02	0.0678	57.6%	1.13	0.76	1.70	0.5462	42.4%
PC aa C36:1	1.11	0.87	1.41	0.6891	1.01	0.75	1.37	0.9395	64.5%	1.31	0.87	1.97	0.2015	35.5%
PC aa C36:2	1.19	0.93	1.53	0.6891	1.09	0.81	1.47	0.5771	68.1%	1.44	0.93	2.24	0.1015	31.9%
PC aa C36:3	1.26	0.99	1.60	0.5556	1.10	0.82	1.48	0.5332	65.7%	1.65	1.09	2.48	0.0170	34.3%
PC aa C36:4	1.14	0.89	1.46	0.6891	0.98	0.71	1.35	0.9021	59.4%	1.42	0.96	2.08	0.0768	40.6%
PC aa C36:5	0.89	0.68	1.15	0.6891	0.83	0.59	1.17	0.2887	56.7%	0.97	0.65	1.45	0.8916	43.3%
PC aa C36:6	0.80	0.60	1.06	0.6891	0.69	0.48	1.00	0.0474	59.3%	1.00	0.64	1.55	0.9937	40.7%
PC aa C38:0	0.86	0.66	1.11	0.6891	0.71	0.51	1.00	0.0486	58.9%	1.12	0.75	1.69	0.5797	41.1%
PC aa C38:1	0.93	0.72	1.19	0.7781	0.78	0.56	1.08	0.1290	58.0%	1.20	0.81	1.76	0.3663	42.0%

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	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
PC aa C38:3	1.33	1.03	1.70	0.4518	1.24	0.91	1.68	0.1697	65.7%	1.51	0.99	2.30	0.0585	34.3%
PC aa C38:4	1.16	0.90	1.49	0.6891	1.09	0.79	1.49	0.6161	63.1%	1.30	0.86	1.98	0.2135	36.9%
PC aa C38:5	0.97	0.75	1.25	0.8617	0.86	0.61	1.20	0.3593	57.5%	1.15	0.78	1.70	0.4760	42.5%
PC aa C38:6	0.95	0.74	1.22	0.8024	0.77	0.55	1.07	0.1233	54.3%	1.23	0.85	1.77	0.2718	45.7%
PC aa C40:2	0.69	0.52	0.91	0.3628	0.63	0.44	0.90	0.0102	63.7%	0.80	0.50	1.27	0.3412	36.3%
PC aa C40:3	0.82	0.63	1.06	0.6891	0.72	0.51	1.02	0.0615	59.6%	0.98	0.65	1.48	0.9147	40.4%
PC aa C40:4	1.13	0.88	1.45	0.6891	1.03	0.75	1.40	0.8755	62.4%	1.33	0.89	1.99	0.1616	37.6%
PC aa C40:5	1.06	0.82	1.37	0.7871	0.90	0.64	1.26	0.5371	55.3%	1.31	0.90	1.91	0.1651	44.7%
PC aa C40:6	1.03	0.80	1.33	0.8617	0.87	0.62	1.21	0.4087	56.6%	1.29	0.88	1.89	0.1871	43.4%
PC aa C42:0	0.79	0.61	1.04	0.6891	0.68	0.48	0.96	0.0267	60.9%	1.01	0.66	1.54	0.9702	39.1%
PC aa C42:1	0.73	0.55	0.96	0.4518	0.56	0.39	0.81	0.0017	57.6%	1.04	0.68	1.59	0.8470	42.4%
PC aa C42:2	0.89	0.69	1.14	0.6891	0.68	0.48	0.97	0.0319	51.3%	1.17	0.82	1.68	0.3875	48.7%
PC aa C42:4	0.99	0.77	1.27	0.9452	0.85	0.62	1.16	0.3008	61.9%	1.28	0.85	1.92	0.2324	38.1%
PC aa C42:5	0.91	0.70	1.18	0.7326	0.76	0.54	1.07	0.1138	56.0%	1.16	0.78	1.71	0.4581	44.0%
PC aa C42:6	0.81	0.62	1.06	0.6891	0.62	0.43	0.89	0.0098	55.8%	1.14	0.76	1.71	0.5352	44.2%
Acyl-alkyl-Phosphatidylcholines														
PC ae C30:0	0.83	0.63	1.10	0.6891	0.73	0.51	1.05	0.0913	59.2%	1.01	0.65	1.57	0.9609	40.8%
PC ae C30:2	0.85	0.64	1.13	0.6891	0.85	0.59	1.22	0.3782	63.3%	0.85	0.53	1.37	0.5063	36.7%
PC ae C32:1	0.76	0.57	1.00	0.5556	0.61	0.42	0.88	0.0087	60.4%	1.04	0.66	1.64	0.8618	39.6%
PC ae C32:2	0.71	0.53	0.95	0.4518	0.62	0.43	0.88	0.0080	66.4%	0.93	0.56	1.55	0.7872	33.6%
PC ae C34:0	0.88	0.67	1.15	0.6891	0.80	0.57	1.12	0.1934	62.9%	1.03	0.67	1.59	0.8989	37.1%
PC ae C34:1	0.95	0.73	1.24	0.8195	0.80	0.57	1.12	0.1991	62.7%	1.27	0.82	1.96	0.2779	37.3%
PC ae C34:2	0.92	0.70	1.22	0.7781	0.88	0.63	1.24	0.4667	65.9%	1.02	0.63	1.63	0.9508	34.1%
PC ae C34:3	0.85	0.64	1.13	0.6891	0.82	0.59	1.14	0.2380	70.5%	0.93	0.56	1.56	0.7932	29.5%
PC ae C36:0	0.84	0.64	1.10	0.6891	0.70	0.48	1.03	0.0694	49.5%	1.00	0.68	1.45	0.9885	50.5%
PC ae C36:1	0.92	0.70	1.19	0.7326	0.81	0.59	1.13	0.2103	66.4%	1.16	0.73	1.84	0.5229	33.6%

Table continued on the next page

	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
PC ae C36:2	0.96	0.72	1.28	0.8617	0.85	0.60	1.20	0.3641	68.0%	1.24	0.75	2.05	0.4078	32.0%
PC ae C36:3	1.00	0.77	1.31	0.9899	0.94	0.68	1.30	0.6911	67.0%	1.15	0.72	1.83	0.5572	33.0%
PC ae C36:4	1.06	0.83	1.36	0.7871	1.01	0.74	1.38	0.9631	62.7%	1.15	0.77	1.73	0.4959	37.3%
PC ae C36:5	0.91	0.70	1.18	0.7326	0.87	0.63	1.20	0.3818	64.3%	1.01	0.65	1.56	0.9775	35.7%
PC ae C38:0	0.83	0.63	1.08	0.6891	0.67	0.47	0.96	0.0272	56.6%	1.09	0.73	1.63	0.6831	43.4%
PC ae C38:1	0.82	0.64	1.07	0.6891	0.80	0.58	1.11	0.1755	64.9%	0.87	0.56	1.34	0.5204	35.1%
PC ae C38:2	0.86	0.65	1.13	0.6891	0.69	0.49	0.98	0.0367	64.2%	1.27	0.80	2.02	0.3082	35.8%
PC ae C38:3	1.09	0.84	1.42	0.7326	0.99	0.72	1.37	0.9719	66.7%	1.33	0.84	2.10	0.2250	33.3%
PC ae C38:4	1.16	0.90	1.50	0.6891	1.07	0.78	1.48	0.6783	61.9%	1.33	0.88	2.01	0.1732	38.1%
PC ae C38:5	1.01	0.79	1.31	0.9253	0.92	0.67	1.27	0.6115	61.4%	1.19	0.79	1.78	0.4102	38.6%
PC ae C38:6	0.85	0.66	1.10	0.6891	0.77	0.56	1.07	0.1201	62.6%	0.99	0.65	1.51	0.9714	37.4%
PC ae C40:1	0.91	0.71	1.17	0.7159	0.75	0.54	1.05	0.0905	57.3%	1.18	0.80	1.73	0.4056	42.7%
PC ae C40:2	0.90	0.69	1.17	0.6891	0.84	0.61	1.17	0.3019	66.4%	1.02	0.65	1.60	0.9428	33.6%
PC ae C40:3	0.93	0.71	1.22	0.7871	0.82	0.59	1.16	0.2628	63.9%	1.17	0.74	1.83	0.5028	36.1%
PC ae C40:4	1.09	0.84	1.42	0.7326	0.95	0.68	1.32	0.7409	61.1%	1.37	0.90	2.09	0.1409	38.9%
PC ae C40:5	0.96	0.73	1.28	0.8617	0.81	0.56	1.18	0.2758	57.3%	1.21	0.79	1.86	0.3811	42.7%
PC ae C40:6	0.84	0.64	1.11	0.6891	0.67	0.48	0.95	0.0250	62.1%	1.22	0.79	1.90	0.3706	37.9%
PC ae C42:1	0.89	0.69	1.15	0.6891	0.79	0.57	1.11	0.1739	61.4%	1.07	0.70	1.62	0.7654	38.6%
PC ae C42:2	0.86	0.66	1.13	0.6891	0.72	0.50	1.02	0.0640	59.1%	1.13	0.74	1.72	0.5781	40.9%
PC ae C42:3	0.89	0.69	1.16	0.6891	0.74	0.53	1.04	0.0856	57.9%	1.15	0.77	1.72	0.4846	42.1%
PC ae C42:4	1.12	0.87	1.45	0.6891	1.02	0.73	1.43	0.8936	59.3%	1.29	0.86	1.93	0.2210	40.7%
PC ae C42:5	0.89	0.69	1.16	0.6891	0.76	0.55	1.06	0.1115	62.2%	1.16	0.76	1.78	0.4873	37.8%
PC ae C44:3	0.81	0.62	1.06	0.6891	0.60	0.42	0.87	0.0065	54.7%	1.15	0.77	1.72	0.4927	45.3%
PC ae C44:4	1.16	0.90	1.49	0.6891	1.06	0.76	1.47	0.7508	60.1%	1.33	0.89	2.00	0.1681	39.9%
PC ae C44:5	0.95	0.74	1.22	0.8024	0.79	0.57	1.10	0.1619	58.7%	1.24	0.84	1.82	0.2884	41.3%
PC ae C44:6	0.88	0.69	1.14	0.6891	0.70	0.50	0.98	0.0351	56.5%	1.20	0.82	1.77	0.3397	43.5%

Table continued on the next page

	Overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
Lyso-Phosphatidylcholines														
lysoPC a C14:0	0.97	0.74	1.26	0.8617	0.86	0.60	1.25	0.4347	53.2%	1.10	0.74	1.63	0.6442	46.8%
lysoPC a C16:0	0.89	0.68	1.16	0.6891	0.77	0.54	1.10	0.1510	55.9%	1.06	0.71	1.59	0.7720	44.1%
lysoPC a C16:1	0.90	0.68	1.19	0.7158	0.65	0.41	1.02	0.0589	39.3%	1.11	0.77	1.59	0.5738	60.7%
lysoPC a C17:0	0.74	0.55	0.99	0.5556	0.67	0.46	0.97	0.0347	62.9%	0.86	0.53	1.40	0.5491	37.1%
lysoPC a C18:0	0.83	0.63	1.09	0.6891	0.82	0.58	1.16	0.2578	64.4%	0.84	0.53	1.34	0.4736	35.6%
lysoPC a C18:1	0.82	0.62	1.09	0.6891	0.73	0.50	1.06	0.0951	56.4%	0.96	0.62	1.47	0.8416	43.6%
lysoPC a C18:2	0.87	0.65	1.16	0.6891	0.89	0.62	1.28	0.5190	63.7%	0.85	0.52	1.37	0.4956	36.3%
lysoPC a C20:3	1.05	0.82	1.35	0.8024	1.03	0.75	1.43	0.8508	60.7%	1.08	0.73	1.62	0.6944	39.3%
lysoPC a C20:4	0.83	0.64	1.09	0.6891	0.82	0.58	1.16	0.2619	58.6%	0.85	0.56	1.29	0.4536	41.4%
lysoPC a C28:1	0.74	0.54	1.00	0.5556	0.72	0.49	1.06	0.0989	61.4%	0.77	0.47	1.26	0.2990	38.6%
Sphingomyelins														
SM C16:0	1.22	0.95	1.57	0.6891	1.08	0.79	1.49	0.6181	63.9%	1.50	0.99	2.28	0.0561	36.1%
SM C16:1	1.29	0.99	1.67	0.5556	1.14	0.84	1.55	0.4097	72.4%	1.77	1.08	2.92	0.0244	27.6%
SM C18:0	1.35	1.06	1.73	0.4518	1.37	1.01	1.86	0.0448	64.8%	1.32	0.87	2.00	0.1932	35.2%
SM C18:1	1.41	1.10	1.80	0.3628	1.36	1.02	1.81	0.0352	73.4%	1.55	0.96	2.49	0.0733	26.6%
SM C20:2	0.92	0.69	1.22	0.7693	0.82	0.57	1.17	0.2664	63.6%	1.12	0.70	1.80	0.6276	36.4%
SM C24:0	1.18	0.92	1.51	0.6891	1.22	0.90	1.65	0.1928	66.3%	1.11	0.73	1.69	0.6361	33.7%
SM C24:1	1.16	0.91	1.48	0.6891	1.06	0.78	1.44	0.7126	62.1%	1.34	0.91	1.99	0.1396	37.9%
SM C26:1	1.13	0.89	1.42	0.6891	1.13	0.84	1.52	0.4110	62.3%	1.12	0.77	1.63	0.5631	37.7%
SM (OH) C14:1	1.04	0.79	1.36	0.8617	1.05	0.76	1.46	0.7555	70.5%	1.00	0.60	1.65	0.9894	29.5%
SM (OH) C16:1	1.13	0.87	1.47	0.6891	1.14	0.83	1.55	0.4246	69.8%	1.13	0.70	1.82	0.6123	30.2%
SM (OH) C22:1	1.14	0.88	1.48	0.6891	1.17	0.86	1.59	0.3069	72.1%	1.05	0.64	1.72	0.8444	27.9%
SM (OH) C22:2	1.12	0.86	1.46	0.6891	1.15	0.85	1.56	0.3536	77.0%	1.03	0.59	1.78	0.9273	23.0%
SM (OH) C24:1	0.95	0.74	1.23	0.8091	1.01	0.74	1. 37	0.9655	70.8%	0.83	0.52	1.34	0.4465	29.2%

Abbreviations: a, acyl; ae, acyl-alkyl; aa, diacyl; PC, phosphatidylcholine; SM, sphingomyelin

[a] Adjusted for age, baseline measures of BMI, waist and hip circumference, smoking status, prevalent and incident diseases including myocardial infarction, stroke, diabetes and any type of cancer

Table 9 Combined fixed-effects meta-analytic and study-specific association of metabolites with the hip-gaining phenotype in men [a]

	overall				EPIC-Potsdam					KORA				
	OR	**LCL**	**UCL**	**P_{FDR}**	**OR**	**LCL**	**UCL**	**uncorrected p value**	**meta weight**	**OR**	**LCL**	**UCL**	**uncorrected p value**	**meta weight**
Amino Acids														
Arginine	0.86	0.66	1.11	0.9943	0.89	0.65	1.22	0.4737	67.5%	0.79	0.50	1.25	0.3084	32.5%
Glutamine	0.94	0.73	1.22	0.9943	0.92	0.67	1.26	0.6045	66.6%	0.98	0.63	1.53	0.9324	33.4%
Glycine	0.87	0.65	1.16	0.9943	1.02	0.74	1.40	0.9244	80.2%	0.47	0.24	0.89	0.0217	19.8%
Histidine	0.84	0.65	1.09	0.9943	0.85	0.61	1.17	0.3045	64.5%	0.84	0.54	1.29	0.4257	35.5%
Methionine	0.79	0.61	1.03	0.9943	0.85	0.61	1.19	0.3411	62.6%	0.71	0.46	1.09	0.1161	37.4%
Ornithine	0.73	0.54	0.98	0.9943	0.79	0.56	1.11	0.1785	72.3%	0.59	0.34	1.03	0.0649	27.7%
Phenylalanine	0.88	0.68	1.13	0.9943	1.01	0.75	1.36	0.9442	75.1%	0.57	0.34	0.96	0.0331	24.9%
Proline	1.04	0.82	1.32	0.9943	1.05	0.78	1.41	0.7532	66.0%	1.01	0.67	1.53	0.9480	34.0%
Serine	0.75	0.56	1.00	0.9943	0.72	0.50	1.03	0.0732	65.8%	0.81	0.49	1.33	0.4053	34.2%
Threonine	0.83	0.64	1.08	0.9943	0.76	0.54	1.07	0.1190	60.0%	0.93	0.61	1.41	0.7376	40.0%
Tryptophan	0.85	0.66	1.09	0.9943	0.82	0.60	1.11	0.1981	64.5%	0.92	0.61	1.40	0.7092	35.5%
Tyrosine	0.87	0.67	1.13	0.9943	0.96	0.69	1.33	0.8022	64.9%	0.73	0.47	1.13	0.1569	35.1%
Valine	0.83	0.63	1.09	0.9943	0.96	0.69	1.33	0.7936	69.9%	0.59	0.36	0.98	0.0414	30.1%
Hexose														
H1	1.13	0.85	1.49	0.9943	1.09	0.78	1.51	0.6262	72.8%	1.26	0.74	2.15	0.4035	27.2%
Acylcarnitines														
C0	1.06	0.82	1.36	0.9943	1.04	0.76	1.41	0.8165	67.3%	1.11	0.71	1.73	0.6436	32.7%
C2	1.04	0.83	1.29	0.9943	1.01	0.78	1.30	0.9455	75.8%	1.13	0.72	1.77	0.5830	24.2%
C3	1.09	0.85	1.40	0.9943	1.23	0.92	1.65	0.1680	70.6%	0.82	0.52	1.29	0.3796	29.4%
C5-OH (C3-DC-M)	1.05	0.83	1.32	0.9943	1.07	0.81	1.42	0.6369	66.2%	1.00	0.67	1.48	0.9842	33.8%
C7-DC	1.04	0.82	1.31	0.9943	1.05	0.78	1.40	0.7591	65.9%	1.02	0.68	1.53	0.9332	34.1%
C9	1.05	0.82	1.33	0.9943	1.03	0.77	1.39	0.8400	66.1%	1.08	0.71	1.64	0.7169	33.9%
C10	0.95	0.75	1.22	0.9943	0.91	0.67	1.22	0.5173	66.6%	1.05	0.69	1.60	0.8109	33.4%
C10:2	0.91	0.71	1.17	0.9943	0.83	0.61	1.13	0.2303	64.1%	1.09	0.72	1.65	0.6842	35.9%

Table continued on the next page

	overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
C14:1	1.02	0.80	1.29	0.9943	0.99	0.73	1.34	0.9301	60.5%	1.06	0.73	1.55	0.7476	39.5%
C14:2	0.98	0.78	1.23	0.9943	0.86	0.63	1.17	0.3361	56.7%	1.15	0.81	1.64	0.4246	43.3%
C16	1.08	0.84	1.38	0.9943	1.07	0.80	1.44	0.6552	69.5%	1.11	0.71	1.73	0.6530	30.5%
C18	1.33	1.04	1.69	0.9943	1.50	1.12	2.00	0.0063	69.5%	1.02	0.66	1.57	0.9448	30.5%
C18:1	1.30	1.04	1.63	0.9943	1.40	1.06	1.85	0.0167	65.8%	1.12	0.76	1.65	0.5586	34.2%
C18:2	1.16	0.92	1.47	0.9943	1.26	0.95	1.66	0.1072	69.9%	0.97	0.63	1.48	0.8728	30.1%
Diacyl-Phosphatidylcholines														
PC aa C28:1	1.17	0.91	1.50	0.9943	1.35	1.01	1.78	0.0395	76.4%	0.75	0.45	1.24	0.2555	23.6%
PC aa C30:0	1.01	0.79	1.29	0.9943	1.14	0.86	1.51	0.3545	76.8%	0.68	0.41	1.13	0.1352	23.2%
PC aa C32:0	1.00	0.78	1.28	0.9958	1.03	0.75	1.41	0.8500	62.9%	0.95	0.63	1.43	0.7973	37.1%
PC aa C32:1	0.94	0.73	1.21	0.9943	1.01	0.72	1.42	0.9564	57.3%	0.85	0.57	1.26	0.4121	42.7%
PC aa C32:2	1.10	0.86	1.40	0.9943	1.20	0.92	1.58	0.1814	81.9%	0.72	0.41	1.28	0.2615	18.1%
PC aa C32:3	0.98	0.75	1.29	0.9943	0.96	0.70	1.32	0.8040	72.7%	1.04	0.62	1.75	0.8913	27.3%
PC aa C34:1	0.95	0.74	1.22	0.9943	0.94	0.69	1.28	0.7102	64.2%	0.96	0.63	1.45	0.8454	35.8%
PC aa C34:2	1.04	0.81	1.34	0.9943	0.98	0.71	1.34	0.8846	63.1%	1.17	0.77	1.76	0.4578	36.9%
PC aa C34:3	1.00	0.77	1.30	0.9943	1.06	0.78	1.45	0.7123	69.2%	0.89	0.55	1.41	0.6113	30.8%
PC aa C34:4	1.04	0.80	1.35	0.9943	1.18	0.87	1.59	0.2923	73.8%	0.73	0.44	1.22	0.2328	26.2%
PC aa C36:0	0.97	0.75	1.25	0.9943	0.96	0.70	1.33	0.8218	66.0%	0.98	0.63	1.52	0.9198	34.0%
PC aa C36:1	0.92	0.71	1.19	0.9943	0.96	0.70	1.30	0.7764	69.7%	0.85	0.53	1.36	0.4934	30.3%
PC aa C36:2	1.03	0.80	1.33	0.9943	0.95	0.70	1.31	0.7649	65.9%	1.20	0.77	1.85	0.4252	34.1%
PC aa C36:3	1.09	0.85	1.40	0.9943	1.07	0.79	1.46	0.6617	63.7%	1.12	0.75	1.69	0.5754	36.3%
PC aa C36:4	1.06	0.84	1.34	0.9943	1.11	0.83	1.50	0.4864	61.8%	0.98	0.67	1.44	0.9346	38.2%
PC aa C36:5	0.90	0.68	1.18	0.9943	1.00	0.73	1.37	0.9996	73.8%	0.66	0.39	1.13	0.1326	26.2%
PC aa C36:6	0.97	0.74	1.27	0.9943	1.11	0.81	1.51	0.5226	75.2%	0.66	0.38	1.13	0.1290	24.8%
PC aa C38:0	0.87	0.66	1.15	0.9943	0.85	0.61	1.20	0.3630	66.2%	0.90	0.56	1.45	0.6674	33.8%
PC aa C38:1	0.74	0.56	0.98	0.9943	0.67	0.47	0.97	0.0326	59.9%	0.86	0.55	1.34	0.5098	40.1%

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	overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
PC aa C38:3	1.07	0.82	1.38	0.9943	1.09	0.79	1.52	0.5946	62.0%	1.03	0.68	1.56	0.9017	38.0%
PC aa C38:4	1.05	0.83	1.33	0.9943	1.09	0.81	1.47	0.5726	63.4%	0.99	0.67	1.47	0.9759	36.6%
PC aa C38:5	0.99	0.78	1.26	0.9943	1.05	0.78	1.42	0.7479	64.1%	0.89	0.60	1.33	0.5736	35.9%
PC aa C38:6	0.97	0.76	1.24	0.9943	0.98	0.72	1.34	0.8940	62.9%	0.96	0.64	1.44	0.8290	37.1%
PC aa C40:2	0.78	0.59	1.04	0.9943	0.72	0.50	1.03	0.0751	61.7%	0.89	0.56	1.40	0.6048	38.3%
PC aa C40:3	0.85	0.65	1.13	0.9943	0.70	0.48	1.04	0.0739	51.5%	1.05	0.71	1.57	0.8009	48.5%
PC aa C40:4	1.10	0.88	1.38	0.9943	1.07	0.79	1.44	0.6698	58.3%	1.16	0.82	1.65	0.4098	41.7%
PC aa C40:5	1.06	0.84	1.34	0.9943	1.11	0.83	1.49	0.4919	63.1%	0.99	0.68	1.46	0.9752	36.9%
PC aa C40:6	0.96	0.75	1.24	0.9943	0.99	0.72	1.36	0.9374	62.6%	0.92	0.61	1.40	0.7005	37.4%
PC aa C42:0	1.05	0.80	1.36	0.9943	1.10	0.80	1.51	0.5509	70.1%	0.93	0.57	1.51	0.7653	29.9%
PC aa C42:1	1.16	0.90	1.49	0.9943	1.26	0.94	1.70	0.1211	73.5%	0.91	0.56	1.49	0.7031	26.5%
PC aa C42:2	1.07	0.83	1.37	0.9943	1.07	0.79	1.45	0.6633	65.3%	1.06	0.70	1.61	0.7900	34.7%
PC aa C42:4	0.84	0.65	1.10	0.9943	0.90	0.66	1.23	0.5118	68.8%	0.73	0.46	1.17	0.1917	31.2%
PC aa C42:5	0.86	0.66	1.13	0.9943	0.77	0.54	1.09	0.1438	57.8%	1.01	0.67	1.53	0.9627	42.2%
PC aa C42:6	1.08	0.84	1.38	0.9943	1.14	0.83	1.55	0.4230	62.6%	0.99	0.66	1.48	0.9607	37.4%
Acyl-alkyl-Phosphatidylcholines														
PC ae C30:0	1.05	0.82	1.35	0.9943	1.18	0.89	1.56	0.2531	81.7%	0.64	0.35	1.15	0.1339	18.3%
PC ae C30:2	1.23	0.97	1.55	0.9943	1.27	0.96	1.68	0.0914	71.2%	1.12	0.72	1.74	0.6040	28.8%
PC ae C32:1	0.91	0.71	1.19	0.9943	0.97	0.72	1.32	0.8670	72.4%	0.78	0.47	1.27	0.3131	27.6%
PC ae C32:2	0.88	0.67	1.15	0.9943	0.91	0.67	1.26	0.5764	73.7%	0.79	0.46	1.34	0.3803	26.3%
PC ae C34:0	1.03	0.80	1.32	0.9943	1.16	0.86	1.55	0.3323	75.5%	0.71	0.43	1.19	0.1970	24.5%
PC ae C34:1	1.00	0.77	1.29	0.9943	1.08	0.81	1.44	0.6054	78.5%	0.75	0.43	1.30	0.3069	21.5%
PC ae C34:2	0.93	0.72	1.22	0.9943	1.02	0.75	1.38	0.9033	76.7%	0.70	0.40	1.21	0.1998	23.3%
PC ae C34:3	0.92	0.69	1.21	0.9943	0.97	0.70	1.34	0.8494	73.7%	0.78	0.45	1.35	0.3710	26.3%
PC ae C36:0	0.82	0.64	1.06	0.9943	0.84	0.61	1.15	0.2635	63.1%	0.80	0.53	1.21	0.2925	36.9%
PC ae C36:1	1.06	0.82	1.37	0.9943	1.20	0.90	1.60	0.2207	77.6%	0.70	0.41	1.21	0.2025	22.4%

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	overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
PC ae C36:2	1.02	0.78	1.34	0.9943	1.05	0.77	1.43	0.7470	76.1%	0.93	0.54	1.62	0.7993	23.9%
PC ae C36:3	0.88	0.67	1.16	0.9943	0.94	0.69	1.29	0.6982	74.9%	0.73	0.42	1.25	0.2476	25.1%
PC ae C36:4	1.00	0.78	1.27	0.9943	1.07	0.80	1.44	0.6422	68.3%	0.85	0.56	1.31	0.4720	31.7%
PC ae C36:5	0.94	0.74	1.21	0.9943	1.00	0.74	1.37	0.9826	63.8%	0.85	0.56	1.28	0.4391	36.2%
PC ae C38:0	0.94	0.73	1.22	0.9943	1.01	0.74	1.38	0.9693	68.5%	0.82	0.52	1.31	0.4044	31.5%
PC ae C38:1	0.99	0.78	1.27	0.9943	1.00	0.75	1.34	0.9874	70.1%	0.98	0.63	1.53	0.9402	29.9%
PC ae C38:2	0.98	0.75	1.28	0.9943	0.95	0.70	1.30	0.7550	74.5%	1.06	0.62	1.81	0.8321	25.5%
PC ae C38:3	1.11	0.86	1.43	0.9943	1.25	0.94	1.67	0.1291	76.0%	0.76	0.45	1.27	0.2906	24.0%
PC ae C38:4	1.00	0.78	1.28	0.9943	1.06	0.79	1.42	0.7105	68.9%	0.89	0.57	1.38	0.6082	31.1%
PC ae C38:5	0.96	0.75	1.23	0.9943	0.97	0.71	1.31	0.8177	66.1%	0.94	0.62	1.44	0.7890	33.9%
PC ae C38:6	0.89	0.68	1.16	0.9943	0.94	0.68	1.30	0.6966	68.3%	0.79	0.49	1.28	0.3356	31.7%
PC ae C40:1	0.95	0.75	1.22	0.9943	0.99	0.73	1.34	0.9606	66.0%	0.89	0.58	1.35	0.5722	34.0%
PC ae C40:2	0.97	0.75	1.25	0.9943	1.05	0.77	1.42	0.7666	72.1%	0.79	0.48	1.28	0.3297	27.9%
PC ae C40:3	1.01	0.78	1.31	0.9943	1.11	0.83	1.49	0.4823	76.0%	0.76	0.45	1.28	0.2963	24.0%
PC ae C40:4	0.94	0.73	1.21	0.9943	0.96	0.71	1.30	0.7930	70.1%	0.89	0.56	1.42	0.6335	29.9%
PC ae C40:5	0.96	0.74	1.24	0.9943	0.95	0.70	1.31	0.7688	68.3%	0.96	0.60	1.53	0.8646	31.7%
PC ae C40:6	0.96	0.73	1.25	0.9943	0.99	0.72	1.36	0.9295	70.5%	0.90	0.55	1.47	0.6758	29.5%
PC ae C42:1	1.15	0.91	1.45	0.9943	1.12	0.84	1.50	0.4314	64.3%	1.19	0.80	1.75	0.3937	35.7%
PC ae C42:2	1.02	0.79	1.31	0.9943	1.10	0.82	1.49	0.5287	71.4%	0.83	0.52	1.33	0.4403	28.6%
PC ae C42:3	0.95	0.74	1.23	0.9943	0.98	0.71	1.35	0.8960	65.5%	0.90	0.58	1.40	0.6473	34.5%
PC ae C42:4	1.01	0.79	1.29	0.9943	1.06	0.78	1.42	0.7200	70.1%	0.90	0.57	1.43	0.6670	29.9%
PC ae C42:5	0.93	0.71	1.22	0.9943	0.93	0.68	1.29	0.6758	71.2%	0.92	0.56	1.52	0.7384	28.8%
PC ae C44:3	1.13	0.88	1.45	0.9943	1.30	0.97	1.74	0.0830	71.6%	0.81	0.51	1.29	0.3653	28.4%
PC ae C44:4	0.96	0.74	1.23	0.9943	0.98	0.72	1.32	0.8870	69.7%	0.91	0.58	1.44	0.6917	30.3%
PC ae C44:5	1.00	0.77	1.28	0.9943	1.01	0.75	1.36	0.9644	70.3%	0.97	0.61	1.54	0.9015	29.7%
PC ae C44:6	1.06	0.82	1.37	0.9943	1.16	0.85	1.57	0.3547	70.0%	0.87	0.55	1.39	0.5695	30.0%

Table continued on the next page

	overall				EPIC-Potsdam					KORA				
	OR	LCL	UCL	P_{FDR}	OR	LCL	UCL	uncorrected p value	meta weight	OR	LCL	UCL	uncorrected p value	meta weight
Lyso-Phosphatidylcholines														
lysoPC a C14:0	0.93	0.75	1.15	0.9943	1.00	0.79	1.27	0.9973	79.7%	0.70	0.43	1.13	0.1407	20.3%
lysoPC a C16:0	0.90	0.70	1.15	0.9943	0.96	0.72	1.30	0.8094	69.1%	0.77	0.50	1.20	0.2521	30.9%
lysoPC a C16:1	0.93	0.74	1.18	0.9943	1.05	0.78	1.42	0.7313	63.9%	0.76	0.51	1.12	0.1646	36.1%
lysoPC a C17:0	1.03	0.80	1.34	0.9943	1.13	0.84	1.52	0.4161	74%	0.80	0.49	1.33	0.3889	26%
lysoPC a C18:0	0.92	0.71	1.20	0.9943	0.97	0.71	1.33	0.8523	69.1%	0.82	0.51	1.32	0.4192	30.9%
lysoPC a C18:1	0.90	0.71	1.15	0.9943	0.93	0.68	1.27	0.6606	62.7%	0.85	0.57	1.27	0.4372	37.3%
lysoPC a C18:2	0.99	0.75	1.30	0.9943	1.05	0.76	1.45	0.7629	70.5%	0.85	0.52	1.40	0.5292	29.5%
lysoPC a C20:3	1.02	0.80	1.30	0.9943	1.18	0.87	1.60	0.2810	65.1%	0.77	0.51	1.16	0.2075	34.9%
lysoPC a C20:4	0.98	0.78	1.25	0.9943	1.07	0.80	1.45	0.6376	64%	0.84	0.57	1.25	0.4002	36%
lysoPC a C28:1	1.05	0.83	1.32	0.9943	1.07	0.82	1.39	0.6207	75.4%	1.00	0.63	1.59	0.9871	24.6%
Sphingomyelins														
SM C16:0	0.95	0.74	1.22	0.9943	0.93	0.69	1.26	0.6437	69.4%	0.99	0.64	1.56	0.9824	30.6%
SM C16:1	1.05	0.81	1.36	0.9943	1.06	0.78	1.43	0.7190	74.5%	1.02	0.61	1.71	0.9415	25.5%
SM C18:0	0.98	0.76	1.26	0.9943	0.94	0.69	1.28	0.7107	66.2%	1.05	0.69	1.62	0.8131	33.8%
SM C18:1	0.99	0.75	1.29	0.9943	0.91	0.66	1.26	0.5727	69.2%	1.17	0.73	1.90	0.5141	30.8%
SM C20:2	0.90	0.67	1.21	0.9943	0.81	0.56	1.18	0.2757	63.1%	1.06	0.65	1.73	0.8140	36.9%
SM C24:0	0.90	0.67	1.21	0.9943	1.08	0.81	1.44	0.6147	69.7%	0.72	0.46	1.11	0.1388	30.3%
SM C24:1	0.95	0.75	1.21	0.9943	0.85	0.62	1.16	0.3075	66.2%	1.01	0.65	1.56	0.9617	33.8%
SM C26:1	0.90	0.70	1.16	0.9943	0.98	0.71	1.34	0.8888	66.5%	0.83	0.53	1.29	0.4101	33.5%
SM (OH) C14:1	0.93	0.72	1.20	0.9943	1.18	0.89	1.57	0.2455	78.0%	0.73	0.43	1.24	0.2456	22.0%
SM (OH) C16:1	1.06	0.83	1.37	0.9943	1.03	0.76	1.38	0.8668	72.4%	0.93	0.57	1.50	0.7532	27.6%
SM (OH) C22:1	1.00	0.78	1.28	0.9943	1.09	0.82	1.47	0.5464	74.6%	0.65	0.39	1.08	0.0942	25.4%
SM (OH) C22:2	0.96	0.74	1.23	0.9943	1.07	0.79	1.46	0.6519	77.1%	0.69	0.39	1.22	0.2048	22.9%
SM (OH) C24:1	0.97	0.74	1.27	0.9943	1.19	0.89	1.59	0.2454	75.0%	0.62	0.38	1.03	0.0636	25.0%

Abbreviations: a, acyl; ae, acyl-alkyl; aa, diacyl; PC, phosphatidylcholine; SM, sphingomyelin

[a] Adjusted for age, baseline measures of BMI, waist and hip circumference, smoking status, prevalent and incident diseases including myocardial infarction, stroke, diabetes and any type of cancer

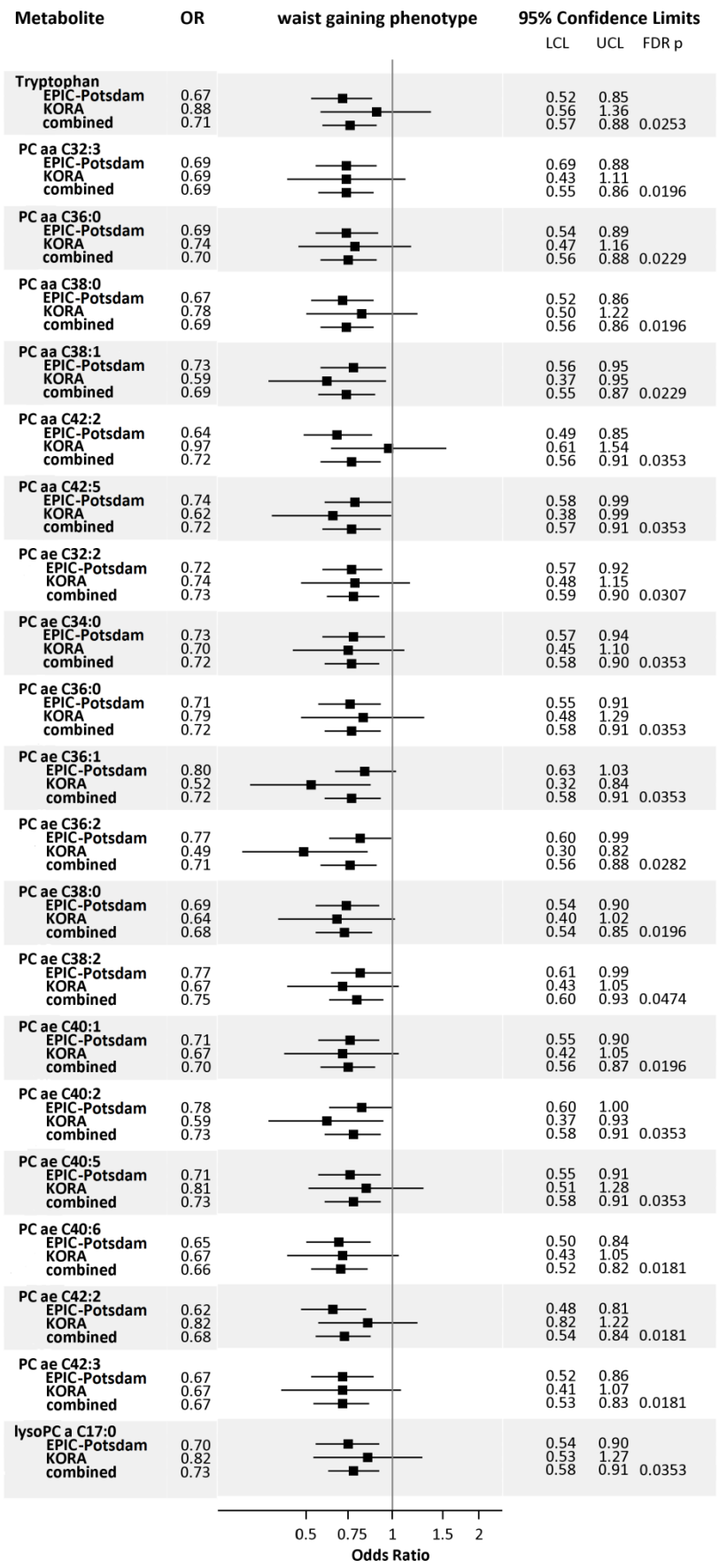

Figure 12 Forest plot of metabolites showing statistically significant combined association with WG phenotype in women

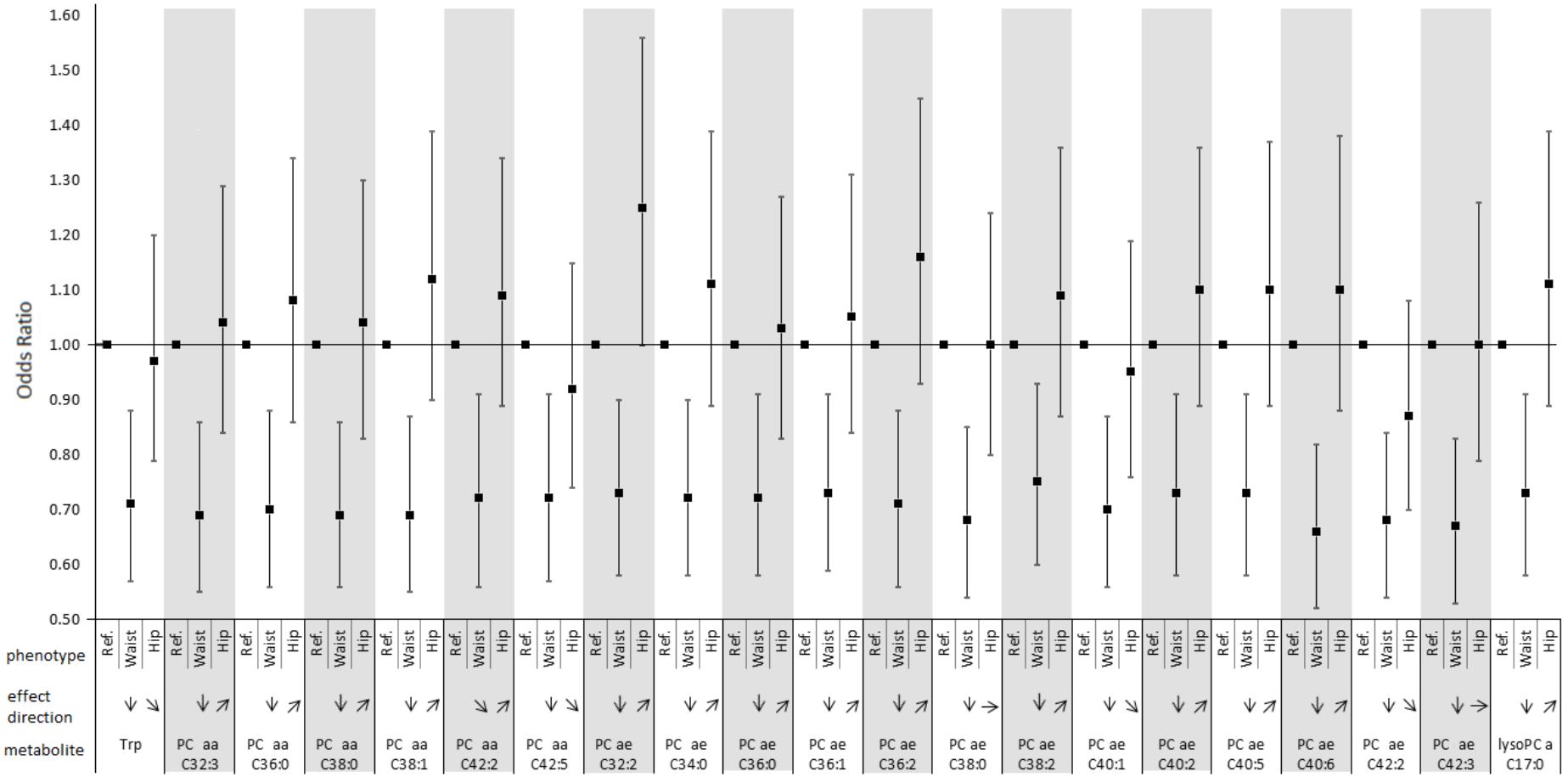

Figure 13 Combined estimates and corresponding confidence limits of selected metabolites with waist and hip-gaining phenotypes in women

4.3.5. Correlations between metabolites and anthropometric measures

The majority of quality-controlled metabolites showed different correlations with cross-sectional anthropometric measures compared to longitudinal changes of these measures. In general, correlations were weak in both sexes. Strongest negative correlations between anthropometric markers and metabolite concentrations were observed between baseline BMI and lysoPC C18:2 in men ($r=-0.31$, $p<.0001$) and baseline WHR with acyl-alkyl-PC C34:3 in women ($r=-0.34$, $p<.0001$). Strongest positive correlations were observed between baseline BMI and diacyl-PC C38:3 in men ($r=0.34$, $p<.0001$) and baseline waist circumference with the same metabolite in women ($r=0.37$, $p<.0001$). All other metabolites showed correlations within this range.

Metabolites that showed statistically significant associations with the WG phenotype in women are shown in Figure 14. For these metabolites, values ranged from $r=-0.28$ ($p<.0001$) for acyl-alkyl-PC C42:3 with baseline waist circumference to $r=0.13$ ($p<.0001$) for the same metabolite with average annual change in waist circumference; for men values ranged from $r=-0.23$ ($p<.0001$) for lysoPC C17:0 with baseline waist circumference to $r=0.15$ ($p<.0001$) for diacyl-PC C42:5 with baseline waist circumference. For these selected metabolites, women showed in general stronger correlations compared to men, but comparable directions in their correlation.

Most of the PCs showed inverse cross-sectional correlations with anthropometric markers at baseline indicated by a blue colour in the corresponding heat map whereas the average annual changes of those measures showed positive associations indicated by a red colour in the corresponding heat map (Figure 14).

Aromatic AA tryptophan showed comparable results in both sexes for both perspectives. Cross-sectional and longitudinal anthropometric measures did not show statistically significant correlations (range $r=-0.01$ to $r=0.03$).

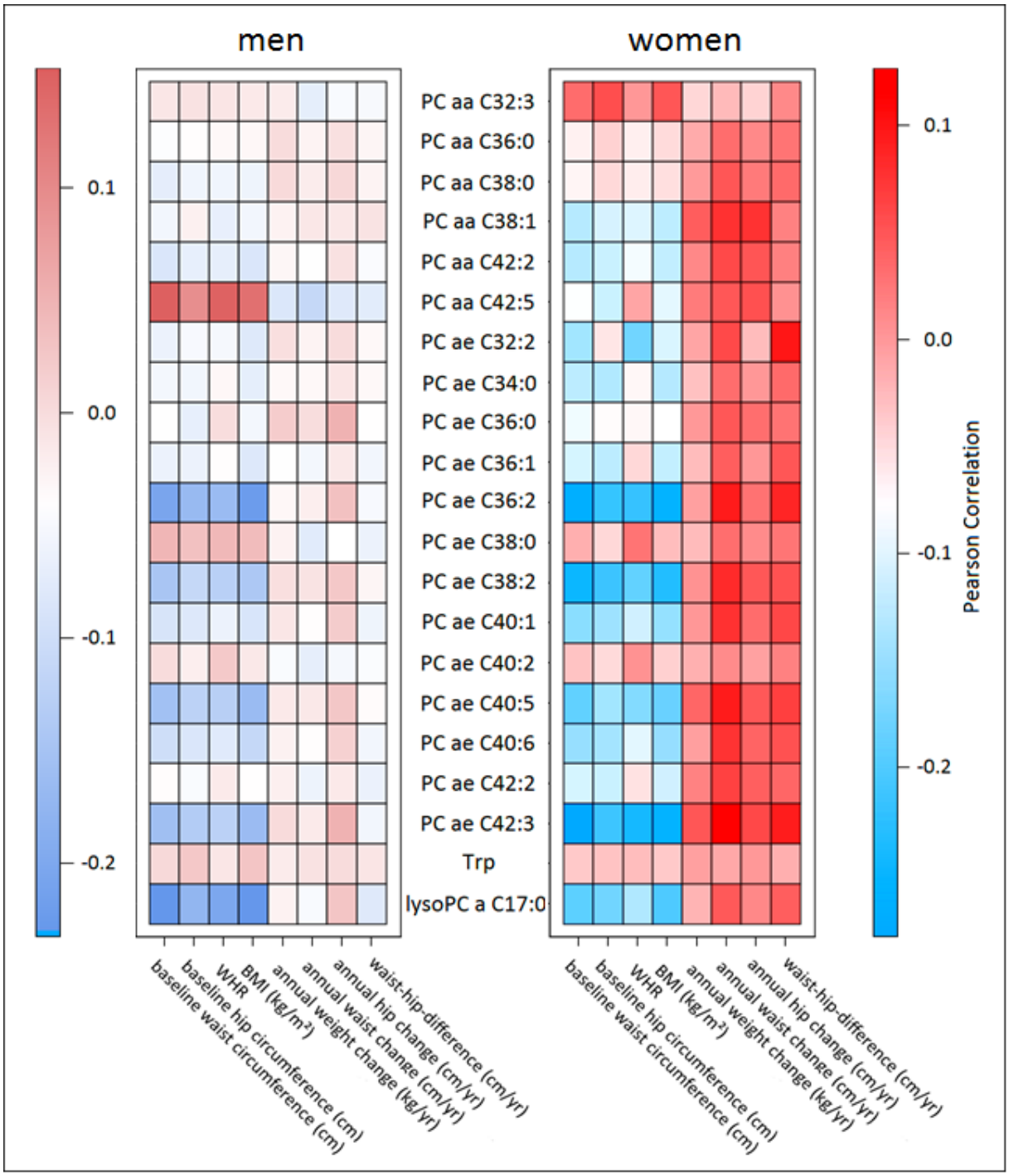

Figure 14 Heat Map showing cross-sectional and longitudinal anthropometric markers and their correlation to selected metabolites for men and women

4.4. Comparing the results of two different approaches (objective 3)

After conducting sex-specific PCA with the metabolite data, five principal components for men and four principal components for women were required to account for the majority of explained variance. The five derived principal components in men explained 55.0% of the total variance for men; the four principal components in women explained 50.1% of the total variance for women (Figure 15). After orthogonal varimax rotation, rotated components accounted for 14.3%, 14.3%, 12.1%, 8.0% and 6.3% of the total variance in men; rotated components accounted for 17.1%, 15.3%, 10.0% and 7.8% of the total variance in women.

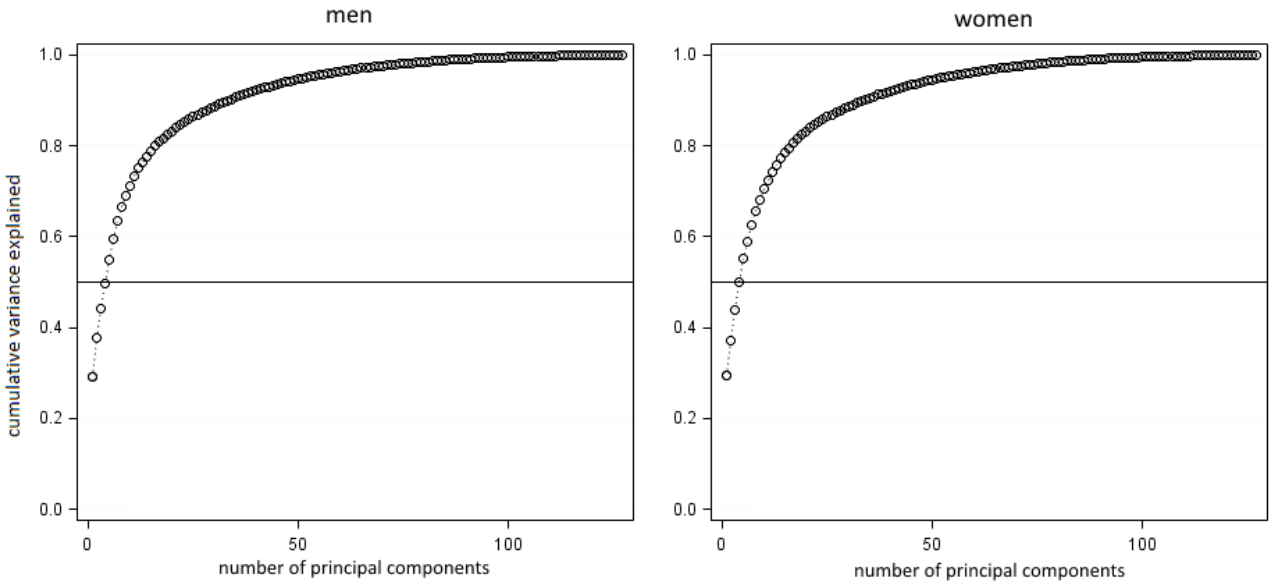

Figure 15 Cumulative variance explained by increasing number of principal components

The composition for each principal component regarding metabolites with meaningful factor loadings was comparable for men and women (Table 10 & Table 11). Two principal components contained mostly PCs, one component contained AAs and another component in the main SMs. For men, an additional component with ACs was derived. Factor loadings were as well comparable between sexes for most components. Complete rotated factor patterns are shown in supplemental Appendix 6 and Appendix 7.

No metabolite showed meaningful factor loadings on more than one principal component and was uniquely assignable. For both sexes, principal components 1 and 2 contained mostly PCs but were interchanged in their structure (principal component 1 for women was comparable with principal component 2 in men). In general, components were similar but showed small differences e.g. principal component 4 in men contained threonine and serine whereas principal component 4 in women contained proline instead.

Table 10 Principal components including metabolites with meaningful factor loadings derived from 127 metabolites measured with targeted metabolomics approach in men in EPIC-Potsdam

PComp 1	Factor loading	PComp 2	Factor loading	PComp 3	Factor loading	PComp 4	Factor loading	PComp 5	Factor loading
lysoPC a C16:1	75	PC aa C36:0	69	PC aa C28:1	68	Arginine	63	C14:1	70
PC aa C30:0	74	PC aa C38:0	80	PC ae C30:0	61	Glutamine	68	C14:2	76
PC aa C32:0	73	PC aa C40:2	71	PC ae C36:1	64	Histidine	76	C16:2	65
PC aa C32:1	82	PC aa C40:3	68	PC ae C38:3	62	Methionine	79	C18:1	63
PC aa C32:2	75	PC aa C42:0	84	PC ae C40:2	63	Ornithine	70	C18:2	61
PC aa C34:1	84	PC aa C42:1	81	SM (OH) C14:1	85	Phenylalanine	75	C5-DC / C6-OH	63
PC aa C34:3	78	PC aa C42:2	74	SM (OH) C16:1	89	Serine	63	C7-DC	69
PC aa C34:4	83	PC ae C32:2	62	SM (OH) C22:1	84	Threonine	65		
PC aa C36:1	82	PC ae C38:0	64	SM (OH) C22:2	84	Tryptophan	77		
PC aa C36:3	75	PC ae C38:6	73	SM (OH) C24:1	80	Tyrosine	67		
PC aa C36:4	68	PC ae C40:1	63	SM C16:0	75	Valine	72		
PC aa C36:6	64	PC ae C40:5	73	SM C16:1	66	xLeucine	77		
PC aa C38:3	71	PC ae C40:6	77	SM C18:0	73				
PC aa C38:4	62	PC ae C42:2	65	SM C18:1	68				
PC aa C38:5	76	PC ae C42:3	79	SM C24:0	62				
PC aa C40:4	74	PC ae C42:5	70	SM C26:0	69				
PC aa C40:5	81	PC ae C44:6	69						
PC aa C42:6	62								

Abbreviations: PComp, Principal Component; PC, phosphatidylcholine; a, acyl; e,-alkyl; SM, sphingomyelin

Table 11 Principal components including metabolites with meaningful factor loadings derived from 127 metabolites measured with targeted metabolomics approach in women in EPIC-Potsdam

PComp1	Factor loading	PComp 2	Factor loading	PComp 3	Factor loading	PComp 4	Factor loading
PC aa C36:0	67	PC aa C30:0	77	C14:1	70	Arginine	66
PC aa C38:0	76	PC aa C32:0	75	C14:2	64	Glutamine	66
PC aa C42:0	83	PC aa C32:1	80	C7-DC	61	Histidine	60
PC aa C42:1	81	PC aa C32:2	74	SM (OH) C14:1	62	Methionine	75
PC aa C42:2	64	PC aa C34:1	84	SM (OH) C16:1	69	Ornithine	68
PC ae C32:1	71	PC aa C34:2	60	SM (OH) C22:1	67	Phenylalanine	74
PC ae C32:2	71	PC aa C34:3	75	SM (OH) C22:2	68	Proline	67
PC ae C34:3	68	PC aa C34:4	85	SM (OH) C24:1	71	Tryptophan	74
PC ae C36:5	63	PC aa C36:1	74	SM C16:0	67	Tyrosine	68
PC ae C38:4	63	PC aa C36:3	78	SM C16:1	69	Valine	70
PC ae C38:5	68	PC aa C36:4	72	SM C18:0	73	xLeucine	72
PC ae C38:6	69	PC aa C36:6	72	SM C18:1	69		
PC ae C40:1	62	PC aa C38:3	73	SM C24:0	61		
PC ae C40:3	71	PC aa C38:4	65	SM C26:0	65		
PC ae C40:4	78	PC aa C38:5	74	SM C26:1	63		
PC ae C40:5	82	PC aa C40:4	73				
PC ae C40:6	77	PC aa C40:5	68				
PC ae C42:2	61	PC ae C34:0	65				
PC ae C42:3	78	PC ae C34:1	66				
PC ae C42:4	76	PC ae C36:1	63				
PC ae C42:5	85	PC ae C38:3	61				
PC ae C44:4	63						
PC ae C44:5	73						
PC ae C44:6	82						

Abbreviations: PComp, Principal Component; PC, phosphatidylcholine; a, acyl; e,-alkyl; SM, sphingomyelin

4.4.1. Single metabolites and their association to WG and HG phenotypes in EPIC-Potsdam

In the single metabolite approach restricted to metabolite data from EPIC-Potsdam, 23 metabolites showed significant associations ($p<0.05$), of which 19 metabolites were associated with the WG phenotype and four metabolites with the HG phenotype. When taking correction for multiple testing into account, no metabolite showed statistically significant associations to any of the phenotypes. In women, 36 metabolites showed significant associations ($p<0.05$), 32 metabolites with the WG phenotype and four metabolites with the HG phenotype. When taking multiple testing into account, six metabolites including aromatic AA tryptophan, diacyl-PCs C38:0, C42:2 and acyl-alkyl-PCs C40:6, C42:2 and lyso-PC C17:0 remained significant in their association regarding the WG phenotype; regarding the HG phenotype no metabolite remained significant. In general, more associations were found for the WG than for the HG phenotype in both sexes. This is consistent with the results of the meta-analytical approach (chapter 4.3.1.)

Table 12 Metabolites showing significant associations ($p<0.05$) with WG or HG phenotype in men of EPIC-Potsdam before taking multiple testing into account

	WG phenotype						HG phenotype				
	OR	**LCL**	**UCL**	**P**	**P_{FDR}**		**OR**	**LCL**	**UCL**	**P**	**P_{FDR}**
Phosphatidylcholines						**Acylcarnitines**					
PC aa C32:3	0.70	0.50	0.98	0.04	0.2980	C18:0	1.50	1.12	2.00	0.01	0.8045
PC aa C36:6	0.69	0.48	1.00	0.05	0.3250	C18:1	1.40	1.06	1.85	0.02	0.9996
PC aa C38:0	0.71	0.51	1.00	0.05	0.3250	**Phosphatidylcholines**					
PC aa C40:2	0.63	0.44	0.90	0.01	0.2162	PC aa C28:1	1.35	1.01	1.78	0.04	0.9996
PC aa C42:0	0.68	0.48	0.96	0.03	0.2980	PC aa C38:1	0.67	0.47	0.97	0.03	0.9996
PC aa C42:1	0.56	0.39	0.81	0.002	0.2138						
PC aa C42:2	0.68	0.48	0.97	0.03	0.2980						
PC aa C42:6	0.62	0.43	0.89	0.01	0.2162						
PC ae C30:1	0.61	0.41	0.92	0.02	0.2980						
PC ae C32:1	0.61	0.42	0.88	0.01	0.2162						
PC ae C32:2	0.62	0.43	0.88	0.01	0.2162						
PC ae C38:0	0.67	0.47	0.96	0.03	0.2980						
PC ae C38:2	0.69	0.49	0.98	0.04	0.2980						
PC ae C40:6	0.67	0.48	0.95	0.03	0.2980						
PC ae C44:3	0.60	0.42	0.87	0.01	0.2162						
PC ae C44:6	0.70	0.50	0.98	0.04	0.2980						
Lyso-Phosphatidylcholines											
lysoPC a C17:0	0.67	0.46	0.97	0.03	0.2980						
Sphingomyelins											
SM C18:0	1.37	1.01	1.86	0.04	0.3250						
SM C18:1	1.36	1.02	1.81	0.04	0.2980						

Abbreviations: PC, phosphatidylcholine; a, acyl; e,-alkyl; SM, sphingomyelin

Table 13 Metabolites showing significant associations (p<0.05) with WG or HG phenotype in women of EPIC-Potsdam before taking multiple testing into account

	WG phenotype					HG phenotype				
	OR	**LCL**	**UCL**	**P**	**P_{FDR}**	**OR**	**LCL**	**UCL**	**P**	**P_{FDR}**
Amino Acids						**Acylcarnitines**				
Glycine	0.78	0.61	1.00	0.05	0.1904	C7-DC	1.27	1.00	1.61	0.05	0.9345
Methionine	0.70	0.52	0.93	0.01	0.1004	C9	1.31	1.03	1.66	0.03	0.9345
Serine	0.75	0.59	0.97	0.03	0.1690	**Phosphatidylcholines**					
Tryptophan	0.67	0.52	0.85	0.001	0.0359*	PC aa C40:6	0.71	0.55	0.93	0.01	0.9345
Phosphatidylcholines						**Sphingomyelins**					
PC aa C32:3	0.69	0.54	0.88	0.003	0.0587	SM C24:0	0.76	0.59	0.98	0.03	0.9345
PC aa C36:0	0.69	0.54	0.89	0.004	0.0610						
PC aa C38:0	0.67	0.52	0.86	0.002	0.0359*						
PC aa C38:1	0.73	0.56	0.95	0.02	0.1099						
PC aa C40:2	0.71	0.52	0.97	0.03	0.1773						
PC aa C40:3	0.75	0.58	0.99	0.04	0.1904						
PC aa C42:0	0.72	0.56	0.92	0.01	0.0728						
PC aa C42:1	0.71	0.55	0.91	0.01	0.0642						
PC aa C42:2	0.64	0.49	0.85	0.002	0.0359*						
PC aa C42:5	0.75	0.58	0.99	0.04	0.1904						
PC ae C32:1	0.79	0.62	1.00	0.05	0.1904						
PC ae C32:2	0.72	0.57	0.92	0.01	0.0642						
PC ae C34:0	0.73	0.57	0.94	0.02	0.1004						
PC ae C36:0	0.71	0.55	0.91	0.01	0.0642						
PC ae C36:2	0.77	0.60	0.99	0.05	0.1904						
PC ae C38:0	0.69	0.54	0.90	0.01	0.0642						
PC ae C38:2	0.77	0.61	0.99	0.04	0.1904						
PC ae C38:6	0.78	0.61	0.99	0.04	0.1904						
PC ae C40:1	0.71	0.55	0.90	0.01	0.0642						
PC ae C40:5	0.71	0.55	0.91	0.01	0.0642						
PC ae C40:6	0.65	0.50	0.84	0.001	0.0359*						
PC ae C42:2	0.62	0.48	0.81	0.0004	0.0359*						
PC ae C42:3	0.67	0.52	0.86	0.002	0.0359*						
lyso-Phosphatidylcholines											
lysoPC a C16:0	0.77	0.60	0.99	0.04	0.1904						
lysoPC a C17:0	0.70	0.54	0.90	0.01	0.0642						
lysoPC a C18:0	0.76	0.60	0.98	0.03	0.1742						
lysoPC a C18:1	0.68	0.52	0.88	0.004	0.0587						
lysoPC a C18:2	0.78	0.61	1.00	0.05	0.1904						

* Statistically significant association after correction for multiple testing
Abbreviations: PC, phosphatidylcholine; a, acyl; e,-alkyl; SM, sphingomyelin

Selected results of multiple logistic regression models for men and women of EPIC-Potsdam are shown in Table 12 and Table 13, respectively. In both sexes, especially PCs (diacyl, acyl-alkyl and lyso) showed inverse associations ($p<0.05$) with the WG phenotype. For men, SMs C18:0 and C18:1 showed positive associations whereas in women AAs were inversely associated with the WG phenotype.

In contrast, it could be observed that higher levels of ACs showed suggestive positive associations with HG phenotype for men and women. Furthermore, PCs showed inconsistent associations with acyl-alkyl-PC C28:1 to be positively and diacyl-PC C38:1 to be inversely associated.

In summary, consistent with the results of the meta-analyses reported in chapter 4.3, metabolites associated with the WG phenotype in women could be identified in EPIC-Potsdam. Six metabolites, including one AA and five PCs, remained statistically significant in their association with the WG phenotype in women when taking multiple testing into account. No metabolic predictors could be identified for the WG phenotype in men or for the HG phenotype in both sexes.

4.4.2. Principal components and their association to WG and HG phenotypes in EPIC-Potsdam

After correction for multiple testing, principal component 1 for women and principal component 2 for men, which had high loadings especially on diacyl- and acyl-alkyl-PCs, showed a statistically significant inverse association with the WG phenotype. All other principal components did not show statistically significant associations with any of the weight-gaining phenotypes. All multiple logistic regressions results with principal components as independent variables for both sexes are shown in Table 14. All other factors showed no statistically significant associations for the WG or for the HG phenotype in men and women.

Table 14 Associations and corresponding 95% confidence limits of metabolite principal components with WG and HG phenotype stratified by sex

		WG phenotype				HG phenotype			
		OR	**95% CI**		**P_{FDR}**	**OR**	**95% CI**		**P_{FDR}**
Men	**PComp1**	0.87	0.62	1.21	0.4924	1.09	0.79	1.49	0.7666
	PComp2	0.57	0.39	0.83	0.0143	0.87	0.62	1.24	0.7583
	PComp3	1.23	0.89	1.68	0.4828	1.18	0.88	1.57	0.7583
	PComp4	1.00	0.72	1.40	0.9893	0.89	0.65	1.21	0.7583
	PComp5	1.18	0.87	1.62	0.4828	1.04	0.78	1.40	0.7858
Women	**PComp1**	0.67	0.51	0.86	0.0181	0.96	0.73	1.26	0.7625
	PComp2	0.98	0.78	1.24	0.8751	0.86	0.67	1.10	0.7625
	PComp3	1.03	0.81	1.31	0.8751	1.05	0.81	1.37	0.7625
	PComp4	0.81	0.64	1.03	0.1774	1.06	0.84	1.34	0.7625

Abbreviations: PComp – Principal Component

Simplified principal components

The simplified components are equivalent to the linear combinations of metabolites shown in Table 10 and Table 11.

The simplified linear combinations had excellent correlations with the initial principal components showing that the explained variance of this component is mainly explained by the reduced number of metabolites. For men, Pearson correlation coefficients for the derived linear combinations ranged from r=0.87 for principal component 5 to r=0.94 for principal component 1 (all p<.0001); for women, correlation coefficients ranged from r=0.87 for principal component 3 to r=0.94 for component 2 (all p<.0001) (Table 15).

Table 15 Pearson correlation coefficients and corresponding p-values for simplified principal components with PCA derived principal components

	Men		Women	
	Pearson´s r	**p**	**Pearson´s r**	**p**
Simplified PComp1 with PComp1	0.94	<.0001	0.93	<.0001
Simplified PComp2 with PComp2	0.91	<.0001	0.94	<.0001
Simplified PComp3 with PComp3	0.92	<.0001	0.87	<.0001
Simplified PComp4 with PComp4	0.91	<.0001	0.89	<.0001
Simplified PComp5 with PComp5	0.87	<.0001		

Abbreviation: PComp – Principal Component

Multiple logistic regression analyses were repeated with the simplified principal components as independent variables (Table 16). In women, it could be observed that simplified principal component 1 which is based on the principal component, consisting mainly of PCs with a statistically significant inverse association to the WG phenotype, remained significantly inverse associated. The strength of the association was markedly attenuated in comparison to the initial principal component (OR: 0.98 95% CI: 0.96-0.99, p_{FDR}=0.0304 vs. OR: 0.67, 95% CI: 0.51-0.86, p_{FDR}=0.0181).

A similar observation was made in men. Simplified principal component 2, based on the principal component consisting of PCs, was markedly attenuated in strength of the association and lost its significance when taking multiple testing into account.

None of the other simplified components showed a statistically significant association with any of the phenotypes in men. All associations were markedly attenuated and approached an OR of 1 in comparison to the basic principal components.

Table 16 Associations of simplified principal components to WG or HG phenotype in EPIC-Potsdam by sex

		WG phenotype				HG phenotype			
		OR	**95% CI**		**P_{FDR}**	**OR**	**95% CI**		**P_{FDR}**
Men	**SPComp1**	0.98	0.95	1.02	0.7044	1.01	0.98	1.04	0.6926
	SPComp2	0.95	0.92	0.99	0.0526	1.00	0.96	1.03	0.8847
	SPComp3	1.01	0.98	1.04	0.7704	1.01	0.98	1.05	0.6926
	SPComp4	0.98	0.93	1.03	0.7044	0.98	0.93	1.02	0.6926
	SPComp5	0.99	0.91	1.09	0.8564	1.03	0.95	1.12	0.6926
Women	**SPComp1**	0.98	0.96	0.99	0.0304	1.00	0.98	1.02	0.7977
	SPComp2	0.99	0.97	1.01	0.5807	0.99	0.97	1.01	0.7977
	SPComp3	1.00	0.97	1.03	0.9167	1.00	0.96	1.03	0.7977
	SPComp4	0.96	0.92	1.00	0.1221	1.01	0.97	1.05	0.7977

Abbreviation: SPComp – Simplified Principal Component

4.4.3. Comparison of results

In the principal component approach, the two principal components showing significant associations with the WG phenotype in men or women, respectively, were characterized by high factor loadings on metabolites that showed significant associations in the meta-analytical single metabolite approach. With diacyl-PCs C36:0, C38:0, C42:2 and acyl-alkyl-PCs C32:2, C38:0, C38:6, C40:1, C40:5, C40:6, C42:2, C42:3, 10 out of 19 PCs with statistically significant inverse associations in the meta-analytical approach in objective 2 (chapter 4.3) showed a meaningful factor loading of greater 0.6 on principal component 2 for men; with the same PCs except of acyl-alkyl-PC C38:6, 9 out of 19 PCs showed as well a meaningful factor loading on principal component 1 for women.

None of the principal components showed a statistically significant association to the HG phenotype in any sex. This is consistent with the single-metabolite approach where no metabolite was statistically significantly associated with the HG phenotype when taking multiple testing into account.

5. Discussion

In this thesis, it was possible to identify weight-gaining individuals with either a tendency to increase rather hip circumference or rather waist circumference in three independent well-described population-based prospective German cohort studies. Furthermore, a general tendency to increase rather waist than hip circumference independent of the initial circumferences was observed. On basis of these observations, two phenotypes of WG and HG individuals were defined with significant tendencies to increase rather waist or hip circumference, respectively. Metabolites of a targeted metabolomics approach were investigated as potential predictors of these phenotypes in two independent cohort studies with a meta-analytical combination of single study results afterwards. 21 predictive metabolites could be identified to be predictive for the WG phenotype in women, including 20 glycerophospholipids and one aromatic AA. No predictive metabolite could be identified for the WG phenotype in men. For the HG phenotype, we could not identify a predictive metabolite out of the applied targeted metabolomics kit, neither in men nor in women. In a further approach to investigate the metabolites in their relation to the identified weight-gaining phenotypes, principal components of metabolites where investigated. For both sexes, a principal component containing especially glycerophospholipids was inversely associated with the WG phenotype. Again, no significant association was observed for the HG phenotype. The metabolites that have been identified in the single metabolite approach in women had as well high factor loadings on the principal components that showed significant inverse associations with the same phenotype in both sexes.

The WG phenotype will lead to an increased waist circumference. This is associated with an increased amount of abdominal adipose tissue which is associated with metabolic consequences and increased risk for several chronic diseases. It was possible to identify predictive metabolites of this phenotype.

The HG phenotype will lead to an increased hip circumference. This is associated with an increased amount of peripheral adipose tissue, which has been associated to have inverse associations to mortality and CVD events. It was not possible to identify predictive biomarker of this phenotype.

To the authors´ knowledge this is the first approach to analyse metabolic predictors of WG and HG phenotypes that have been defined using average annual waist-hip-changes.

The tendency towards a stronger increase of abdominal instead of peripheral body fat was previously reported to be present in both sexes. Mousavi *et al.* [121] reported a tendency towards stronger increase of waist circumference during a 6.6-year follow-up

period. Koskova *et al.* [122] reported an increase and a redistribution of regional body fat in women towards the abdominal area during a 3-year follow-up period. This is in line with the results of this thesis where both sexes showed a tendency to increase rather abdominal than peripheral body fat. Furthermore, Ebrahimi-Mameghani *et al.* [123] reported these changes to be stronger in middle-aged compared to older individuals. This is again in line with the results of this thesis where lowest values of waist-hip difference for men and women could be observed in cohort with the highest mean age (KORA).

Lustgarten *et al.* [89] investigated the associations between metabolites and regional body fat in a cross-sectional study. They observed especially AAs, ACs and their derivates to show different associations regarding thigh and abdominal body fat, respectively. In this thesis, mainly phospholipids and not especially ACs or AAs were associated with the future changes in regional body fat and metabolites showed differences in their associations to cross-sectional and longitudinal anthropometric measures. With regard to these differences, cross-sectional and longitudinal results are hardly comparable. Unfortunately, no study was found investigating the longitudinal changes of regional body fat and their association with serum metabolites.

5.1. Discussion of the methods

Study design and study population

The present thesis is based on data of three prospective German cohort studies. All investigated studies are well-described [93, 96, 98]. Based on the longitudinal design it was possible to investigate determinants and predictive metabolites of future weight gain phenotypes.

The study population consists of people that gained weight during the follow-up period, because of the particular interest in weight gain phenotypes. Thus, the obtained results are not representative for the overall German population despite including the nationwide study of DEGS which was corrected for deviations of the sample to the demographic population structure of Germany as a whole [102]. Nevertheless, the study population for objective 1 included two local and one nationwide study and therefore it is possible to report a suggested informative nationwide trend for the weight-gaining population in Germany based on the investigated studies in this thesis.

Differences in the general characteristics of the study population between cohorts may be due to differences in their age structure. KORA had a comparably high mean age at

baseline examination. Due to the high age, participants of KORA suffered more often from chronic diseases like myocardial infarction, stroke, diabetes and cancer and women were more often postmenopausal compared to DEGS and EPIC-Potsdam. With increasing age, chronic diseases appear more often, the observed differences in the characteristics of the cohorts are therefore plausible, but one should consider potentially altered biological effects due to the high age and should be aware of these differences when interpreting and comparing the results between studies. This potential problem was addressed with the comparison of results from KORA with those of EPIC-Potsdam and DEGS, which had a younger mean age. Independent of their age-structure, it was possible to derive consistent phenotypes in each study and observed general trends regarding changes in waist or hip circumferences were comparable.

The analyses of objective 2 and 3 were based on the assumption, that individual weight gain within the follow-up period appeared on the basis of a comparable metabolic profile as measured at baseline examination. The human plasma metabolome is age-dependent, and follow-up periods in KORA and EPIC-Potsdam were 7.1 and 8.6 years, respectively. Individual weight change is not assignable to a special period within follow-up and metabolic changes may have appeared [73]. This point was covered with a subgroup analysis for age and by including age at baseline as covariate in the analyses to proof that effects are not only due to physiological aging effects and corresponding associations were consistent.

Variable assessment

Possible sources of bias are the self-reported measures of body weight, waist and hip circumference for follow-up in EPIC-Potsdam. In order to correct potential underreporting and measurement errors, this possible bias was addressed with the use of EPIC-specific equations that have been invented in the EPIC-Oxford study [100, 101].

As described in the introduction, the effects of waist and hip circumference should only be interpreted considering each other, because of potentially worsening or attenuating effects. This point was covered by the use of the waist-hip difference, and within the logistic regression models by adjusting for baseline BMI and baseline circumferences.

There were some slight differences in the covariate assessment, e.g. menopausal status or for information on chronic diseases. The differences between EPIC-Potsdam and KORA regarding relative numbers of postmenopausal women were expected due to the comparably high mean age of KORA. Therefore, even with homogeneous covariate assessment, KORA would have had the majority of women in a postmenopausal status and

the heterogeneous covariate assessment was therefore expected to show only little impact. To proof that these variables did not show an impact on the observed results, study-specific sensitivity and subgroup analyses dealing with these variables were performed. The observed results were consistent and robust independent of differences in the covariate assessment.

Fixed- vs. Random-effects model

In the statistical analysis for objective 2, it was assumed that the combined studies of EPIC-Potsdam and KORA were functionally identical (share a standardized analysis plan, study population of German individuals, metabolite measurements performed in the same laboratory) and shared a common true effect size, independent of their different age structure. An alternative approach would have been the use of a random-effects model to combine the single study estimates for objective 2. Within this approach, study weights are more balanced and the larger study of EPIC-Potsdam is assigned a less relative weight compared to KORA [115]. Weights would have included the total variance (sum of within- and between-study variance). The total variance can be expressed in a measure of heterogeneity, which means the variation in true effect sizes [124]. The observed heterogeneity expressed as I^2 and computed with the 'DerSimonianLaird'-method [114] was zero for the majority of identified metabolites. In general the observed heterogeneity was low given by an I^2 of less than 50% (Table 17 and Appendix 5) [125]. Possible sources of heterogeneity could be due to reasons of technical and biological variation. Most of the metabolites with a high value for I^2 showed differing directions in their single study associations and therefore would not have met the criterion of a true effect defined in chapter 3.4.4 at all, where both study estimates had to be consistent in their effect direction.

Table 17 Interpretation of I^2 according to Higgins *et al.* (2003) [116]

heterogeneity	**I^2**
No	0%
Low	25%
Moderate	50%
High	75%

Analytical approaches

A possible drawback of the newly created waist-hip difference could be that the same values may appear in both extrema of weight-gaining individuals, e.g. people experiencing a large increase in waist and hip circumference will have a similar values compared to people experiencing just a little increase in waist and hip circumference. Both cases might be based

on different initial levels of anthropometric measures and therefore represent other biochemical processes. Nevertheless, the aim was to identify individuals with the preferred deposition of body fat at specific sites. The assignment of individuals to the specific endpoint or reference categories was possible without any problem.

For EPIC-Potsdam and KORA, these two phenotypes were considered as the endpoints of sex-specific multiple logistic regression models. Those phenotypes are based on study-specific cut-offs of average annual waist-hip difference. Cut-offs used to categorise continuous variables are in most cases arbitrary. The cut-off for categorisation in this thesis was chosen with the extreme deciles, because for approximately 10% of both sexes in EPIC-Potsdam a negative value for waist-hip difference could be observed. A drawback of taking this relative cut-off is that in each investigated study another absolute cut-off emerged and comparisons across studies may be complicated because they differ in their range and values between studies. These cut-offs are reported in Table 5. Nevertheless, both phenotypes clearly represent a significant tendency of larger increase in waist or hip circumference, respectively, independent of their cut-offs and results are therefore comparable in their meaningfulness of WG or HG tendency although absolute values are different.

The waist-hip difference was stronger correlated with average annual changes of waist circumference in comparison to average annual changes in hip circumference. So, changes of waist circumference showed a greater impact on this difference. This should be considered when interpreting the results.

The difference of the average changes of waist and hip circumferences was used to form the endpoint variables of either the WG or the HG phenotype. The use of average changes has several drawbacks, e.g. changes in circumferences might have appeared at different time points within the follow-up period. Thus, an average does not consider this. Nevertheless, the particular interest focused on android or gynoid phenotypes. With the chosen statistical approach it is undoubtedly possible to identify the general tendencies of individuals to one of those body shapes.

A possible drawback of the PCA is that this method creates principal components to explain the variance of the metabolites. Variation within a metabolome dataset is often due to different ages of individuals [73] and so may not explain necessarily much variation with regard to the outcome of interest [120].

For the interpretation of principal components, only variables with a meaningful factor loading of at least 0.6 should be taken into account [120]. Factor loadings can be interpreted

as some kind of correlation between the original variable and the newly created principal component. Metabolites with high factor loadings explain a large amount of variance within this principal component whereas metabolites with low factor loadings explain only a little amount of variance. Nevertheless, the “low-loading” metabolites are part of the derived linear combination. With increasing number of variables, the amount of variance explained by low-loading metabolites increases and should be considered when interpreting the results. The observed attenuated associations from the simplified principal components underline this point of view.

5.2. Discussion of the results

Description of anthropometric changes in weight-gaining individuals

For Germany, it was observed that a gain in body weight is accompanied with a higher gain in waist than in hip circumference. The highest decile of the difference between average annual change of waist and hip circumference was labelled as the WG phenotype and the lowest decile as the HG phenotype.

When comparing the average annual waist-hip difference between men and women, no significant difference could be observed in KORA, visual examination of the study-specific histograms did not reveal large differences between sexes in all studies. This was not expected because gynoid body fat distribution is reported to be more common in women and android body fat distribution is reported to be more common in men [11, 126]. Therefore, a clearer shift of the histograms was expected with the majority of women showing a tendency to increase more hip circumference in comparison to men.

Evidence exists that metabolic disturbances which come along with body weight gain and abdominal body fat accumulation are not completely reversible. These unfavourable metabolic alterations partly remain unchanged after weight loss as well as changes of body composition and lean body mass [127]. For example, evidence exists that overweight or obese individuals with subsequent weight loss decrease their left ventricular mass which in turn increases their risk of suffering from CVD in the future due to decreased circulation ability [128, 129].

The majority of variance for average annual waist-hip difference could be as well explained be the use of average annual changes of WHR. WHR has been shown to be a good measure of abdominal obesity and body fat distribution in general [25], but it has the drawback of difficult interpretability. Thus, the newly created variable presented in this thesis

is able to provide the same information on changes of body fat distribution but it is easier to interpret because of its expression in absolute changes in cm/yr.

The new variable showed stronger correlations with average annual waist circumference than with average annual hip circumference. Therefore this new variable covers a larger variance explanation from changes in waist circumference than from changes in hip circumference. This could be due to the fact that there is just a greater variation present within the waist variable.

DEGS provided nationwide results and could be taken as the reference especially for the anthropometric results. Regional studies of EPIC-Potsdam and KORA showed comparable distributions of average annual waist-hip difference to DEGS, so it can be ruled out that the observed trend is just regional in the regions of Augsburg or Potsdam. The definition of weight-gaining phenotypes for each study revealed different cut-off-values for each study but consistent in their meaningfulness to the WG or the HG phenotype, respectively.

The observed changes in anthropometric markers resulting in a general tendency to increase rather waist circumference than hip circumference could be an index for physiological aging processes. All investigated studies had a mean age of more than 40 years and follow-up periods ranging from 7.1 years (KORA) to 11.9 years (DEGS). In particular EPIC-Potsdam and DEGS cover an age-range in which many hormonal changes appear in women as well as in men. In men, testosterone production decreases with ongoing age leading to lower levels of free plasma testosterone, which is reported to be cross-sectionally and longitudinally associated with abdominal obesity and visceral fat accumulation [130]. In women, oestrogen is the major hormone responsible for the accumulation of fat in the gluteofemoral area. With menopause and the accompanied decline of oestrogen, strong evidence exists that women redistribute their body fat and increase adipose tissue in the abdominal area [40]. But not only testosterone and oestrogen are involved, furthermore the gonadotropins luteinizing hormone and follicle stimulating hormone are reported as being associated with WHR and therefore body fat distribution [25]. Thus, changes in hormonal balance should be considered in the interpretation of results dealing with anthropometric changes in these age-groups.

Associations observed in the single metabolite approach

In objective 2, the particular interest was whether metabolites can predict either the WG or the HG phenotype. The investigated studies are of an observational design, thus it is not possible to prove biological mechanisms. Nevertheless, the discussion of the results may

give some suggestive ideas about possible affected mechanisms that should be confirmed in experimental studies.

Overall, it was possible to identify metabolites whose concentrations were inversely associated with the WG phenotype in women. In men and for the HG phenotype in women, no significant association could be revealed taking multiple testing into account.

Phosphatidylcholines

In the present thesis, it was possible to identify metabolites whose serum concentrations were associated with the defined phenotypes. It appears that associations with $p<0.05$ were revealed more often for the WG than for the HG and that a particular class of metabolites, the PCs, seems to be involved in subsequent waist gain, at least in women. In men and for the HG phenotype, no significant association between serum metabolite concentrations and subsequent gain in circumferences remained when taking multiple testing into account.

PCs are the most abundant phospholipids and a frequent constituent of lipoproteins in human blood, especially HDL [131] typically contains a saturated fatty acid at sn-1 position (e.g. palmitic acid, C16:0, or stearic acid, C18:0) and a poly-unsaturated fatty acid (PUFA) at sn-2 position [132]. De novo synthesis of PC is based on two pathways, the Cytidindiphsophat-choline (CDP-choline) and the phosphatidylethanolamine N-methyltransferase (PEMT)-pathway. The CDP-choline-pathway as the major pathway requires alimentary choline; the PEMT-pathway is depending on the degradation of existing choline containing molecules, in particular phosphatidylethanolamine (PE) [132].

Selected PCs were significantly inversely associated with the WG phenotype in women, with the majority of PCs showing suggestive inverse association in both men and women. Meta-analytical as well as single study results suggest that this subgroup of metabolites seems to be involved in biochemical pathways that lead to the preferred deposition of body fat in the abdominal area.

As mentioned before, there is strong evidence that in particular altered sex-hormones are responsible for the development of abdominal adipose tissue in both men and women [40, 130, 133]. Borruel *et al.* reported the WHR to be significantly correlated with sexual steroids and gonadotropin concentrations [25]. A linkage between oestrogen, testosterone and altered levels of PCs has been reported in mice [134] but information for humans is scarce. In the rodent models, an administration of oestrogen led to an increased PEMT-enzyme activity, whereas the enzyme activity of the CDP-choline pathway was decreased. On the other hand, administration of testosterone did not show an effect on enzyme activities

[134]. For humans, consistent observations for oestrogen were made by Fischer et *al.* [135] reporting the PEMT-pathway to be induced by this hormone in women. Therefore, this subgroup of metabolites seems to be involved in biochemical pathways that lead to the preferred deposition of body fat in the abdominal area and the suggestive linkage between this class of metabolites and sexual hormones may explain the observed associations in this thesis. In EPIC-Potsdam, no significant correlation could be observed for the majority of the metabolites regarding oestrogen or testosterone levels showing that age-related decline of these sex hormones does not play a leading role in the explanation of the observed metabolite-phenotype associations (Appendix 8).

Lyso-PCs are products of hydrolysis from either diacyl- and acyl-alkyl-PCs and have been reported as being associated with body mass and BMI [136]. Metabolic disorders have been reported as being associated with a reduction in lyso-PC-levels [80, 137]. Obese individuals and individuals with impaired glucose tolerance were reported to have decreased plasma levels, with lyso-PCs C16 and C18:2 to be discriminative biomarkers for normal or impaired glucose tolerance [80]. This is consistent with the results of this thesis, where lyso-PC C17:0 was associated with the WG phenotype in both sexes and remained significant in women when taking multiple testing into account. Additionally lyso-PCs showed e.g. inverse associations ($p<0.05$) with both endpoints in both sexes before taking multiple testing into account. Thus, higher levels of lyso-PCs could be an index of a better health status without obesity- or disease-caused metabolic alterations.

Amino acids

The aromatic AA tryptophan was shown to be inversely associated with the WG phenotype in women. Coskun *et al.* [63] reported lower food intake and weight loss in mice treated with tryptophan. As an element of the serotonin metabolism, this AA may be involved in appetite regulation. Subjects with the WG phenotype showed significantly higher rates of average annual weight change and lower levels of tryptophan. Therefore higher levels of this AA may have a small effect on caloric restriction and might therefore be protective against waist gain by restricting weight gain.

Studies investigated cross-sectional associations of metabolites and regional body fat or anthropometric markers like FFMI, percent lean mass or subcutaneous adipose tissue, linking in particular AAs to these anthropometric markers [87-90].

Tanaka *et al.* [137] reported higher levels of many AA including tryptophan in individuals with higher levels of visceral fat compared to individuals with lower levels of visceral fat. Thus, high baseline levels of waist circumference and therefore visceral fat might

be the reason for high tryptophan levels. Tryptophan showed comparable associations with baseline anthropometric markers compared to average annual changes of these markers (chapter 4.3.5). In comparison, individuals with the WG phenotype tended to have a lower baseline waist circumference in the investigated studies. Multiple logistic regression models were adjusted for baseline levels of waist circumference but baseline levels may affect this association through insufficient adjustment and residual confounding.

As a result of the single metabolite approach, it must be emphasized that associations were only observed for the WG phenotype in women, when taking multiple testing into account. Comparing results of uncorrected single study and meta-analyses results, similar observations can be made with men showing less significant associations with the WG phenotype than women. A previously mentioned point is the lower statistical power for men. Another explanation could be differing effect strengths for men and women. Evidence exists that there is a stronger protective effect of gluteofemoral subcutaneous adipose in women [42-44, 138]. An alternative explanation is that the defined weight gain phenotypes had different cut-offs for average annual waist-hip difference in men and in women which could lead to attenuated effects in men.

Null results regarding the hip-gaining phenotype

It was not possible to identify predictors of the HG phenotype for either sex in the meta-analytical approach when taking multiple testing into account. With regard to the uncorrected (suggestive) associations, much less metabolites were associated with the HG phenotype compared to the WG phenotype in both men and women. This is in line with the literature where the information on predictors of gluteofemoral fat mass is scarce. This may be due to the role of visceral adipose tissue as an endocrine organ [39] which is therefore more likely to find predictive metabolites due to its integration in physiological processes. This role might be lacking in the case of hip fat mass.

Associations observed in the principal component approach

It was possible to summarise similar metabolites in consistent and biologically plausible principal components. To cover the majority of explained variance within the metabolomics dataset, a different numbers of principal components were necessary. Nevertheless, the derived components were comparable between men and women. A principal component that showed a similar linear combination of metabolites in men and women (principal component 2 in men and principal component 1 in women, respectively) was associated with the WG phenotype in both sexes.

The majority of PCs that have been identified as being associated with the WG phenotype in the meta-analytic single metabolite approach reappeared in the principal components that showed statistically significant inverse associations with this phenotype in the PCA approach. So results of both approaches are consistent in a way that specific PCs seem to play a role in this association. To study the importance of these metabolites, simplified linear combinations only including metabolites with meaningful factor loadings as weights were computed. Results of these linear combinations as independent variables in the sex-specific multiple logistic regression models were compared to the results obtained with the use of the original principal components. The associations were strongly attenuated for both sexes and even lost its significance among men when taking multiple testing into account. This could mean that not only these metabolites are responsible for the inverse association but the metabolic fingerprint as a whole. As a consequence single metabolites identified in objective 2 should not be seen independently but in combination with each other and one should always consider associated pathways that are affected from these metabolic constellations.

Nonetheless, PCs seem to play a major role in the deposition of body fat in the abdominal area, because both the single metabolite as well as the principal component approach underlined the role of PCs in the development of abdominal fat deposition.

Phenotype-associated metabolites and their association to chronic disease risk

Further research in the EPIC-Heidelberg study revealed that waist circumference is only moderately associated with visceral fat mass [139]. Currently it could not be ruled out that lower gain in waist circumference is particularly related to lower gain in visceral fat mass that is particularly discussed to be involved in the development of chronic diseases like diabetes, hypertension and CVD [65, 140]. There is some evidence that common pathophysiological processes are involved in the development of this chronic diseases [65, 140] and pathways of glycerophospolipids are affected from several diseases. Floegel *et al.* [82] reported four of the associated PCs from the meta-analyses (acyl-alkyl-PCs C40:5, C40:6, C42:3, lyso-PC C17:0) as being associated with a decreased risk of the development of T2D. In line with that, Pietilainen *et al.* [141] reported insulin-sensitive overweight individuals having higher levels of PCs compared to insulin-resistant overweight individuals. Thus, the deposition of body fat in the abdominal area accompanied with a suggested increase in visceral fat may be an intermediate step linking our identified metabolites with predictive association to the WG phenotype to increased risk for the development for T2D.

Insulin sensitivity and lipoprotein membrane composition are linked with each other especially regarding skeletal muscle tissue [141-144]. Lipoproteins consist of PCs on the

outer and PEs on the inner side of the membrane. With a higher degree of PUFAs in the PC-section, bio-membranes become more fluid especially regarding GLUT4 translocation after insulin stimulation. 11 out of 20 of the PCs that have been identified in the single-metabolite approach have at least 2 double bounds and are therefore eligible for containing PUFAs. Thus, higher levels of these metabolites may have beneficial effects in membrane fluidity and increase insulin-sensitivity.

Independent of the endogenous synthesis pathways (CDP-choline or PEMT), especially diacyl-PCs are necessary for the hepatic secretion of very low density lipoprotein (VLDL) and HDL in the liver [132]. Low levels of HDL and increased levels of VLDL are considered as CVD risk factors and therefore PCs are directly involved in the secretion and clearance of those factors.

Acyl-alkyl-PCs were reported to protect lipoproteins from oxidation through their vinyl-ether-bond [145] and so lower risk of CVD. In this thesis, both types of glycerophospholipids with comparable characteristics regarding chain length and degree of saturation showed negative associations with the WG phenotype. It is possible, that upregulated diacyl-PCs lead to the upregulation of acyl-alkyl-PCs to protect them from oxidation and may explain the comparable characteristics.

Ether-PCs and ether-lipids in general were reported to be decreased in hypertensive compared to normotensive individuals independently of other lipid alterations that are induced by obesity and insulin resistance [131, 146]. In this thesis, especially acyl-alkyl-PCs were associated with the WG phenotype in women when taking multiple testing into account or showed meaningful factor loadings for the principal components which showed inverse associations with the WG phenotype in men and women. Hypertension is a risk factor for chronic diseases and appears more often with increasing BMI. In EPIC-Potsdam and KORA, individuals in the reference category suffered less often from chronic diseases and had lower baseline BMI compared to the defined phenotypes which may explain parts of the observed associations between ether-lipids and the WG phenotype.

It cannot be ruled out that some kind of reverse causation is present here. Are the prevalent unfavourable metabolic changes causing chronic diseases or is the unfavourable metabolic constellation caused by prevalent chronic diseases? This needs to be investigated in future studies.

Phosphatidylcholines and their relation to lifestyle

One of the most important confounders that may influence age-related changes in body composition is physical activity. Visceral adipocytes are more sensitive to lipolytic

stimulation than subcutaneous adipocytes [147] and exercise promotes the mobilization of triglycerides from adipose tissues. This association may attenuate or even prevent the age-related gain in visceral adipose tissue by restricting hyperplasia and/or hypertrophy of adipose tissues [148, 149]. Wientzek *et al.* [76] reported both subclasses of PCs, diacyl and acyl-alkyl, to be positively associated with cardiorespiratory fitness (CRF). 8 out of 21 associated metabolites from the single metabolite approach (PCs diacyl C36:0, C38:0, acyl-alkyl C38:0, C42:3, C42:2, C36:0, C40:6, C40:1) were reported to be positively associated with CRF. Those metabolites could be markers of processes that attenuate or prevent the deposition of adipose tissue in the abdominal area through increased lipolysis induced by higher physical activity.

Cross-sectional results of Jourdan *et al.* [87] showed decreasing chain length and saturation of fatty acids in PCs with increasing FFMI. High levels of FFMI indicate a high level of lean body mass in comparison to fat mass which is in general a favourable condition. In this thesis, associated PCs from the single metabolite approach as well as high loading PCs of the significant associated principal components showed in general a lower number of double bonds with decreasing number of carbons. That would be in line with results from Jourdan *et al.* but on the other hand two of associated PCs (acyl-alkyl C36:2 and C42:3) were reported to be negatively associated with FFMI in their study. Furthermore, these results of Jourdan *et al.* [87] did not exist in obese individuals but in non-obese individuals. They hypothesized that a sedentary lifestyle leads to derangements of skeletal muscle metabolism that favours the development of obesity and metabolic diseases because subjects in better shaped exhibit more lipolysis than less fit subjects [150]. In this thesis, the reference category consists in general of non-obese individuals. Body weight gain in these individuals may be restricted through higher physical activity levels indicated by higher FFMI which was reported as being associated with increased levels of PCs. Thus, the hypothesized pathway of comparably higher lipolysis in more fit subjects could be partly explain the inverse association with the WG phenotype and the associated metabolites that have been reported to be positively associated may be markers of the same processes mentioned in the paragraph before.

Summarising the preceding sections shows that higher levels of PCs were reported as being associated with a favourable health status (non-obese, insulin-sensitive, normotensive, higher CRF). This is in line with the observed results that higher levels of PCs were inversely associated with the WG phenotype. The WG phenotype has a perspective of increased chronic disease risk due to the increase in abdominal fat. In general, this could be an indication of a more health-conscious lifestyle of the reference group (e.g. higher physical activity).

5.3. Strength and limitations

The overall strength of the present thesis was the investigation of future phenotypes in the frame of prospective German cohort studies. The present analyses combined up to three prospective population-based and well described German cohort studies, which are all well-described. Thus, strength of this thesis is the longitudinal design of the investigated studies. Based on the longitudinal design it is possible to investigate determinants and predictive factors of future weight gain phenotypes.

Through the combination of two independent cohort studies it was possible to create a larger sample size which was able to reveal even small effect sizes. For example in the single-study approach it was possible to identify six metabolites to be significantly associated with the WG phenotype in EPIC-Potsdam whereas the meta-analytical combination revealed 21 metabolites to be significantly associated. Furthermore it was possible to replicate the results in each study. Thus one can be sure that observed associations are not study-specific and therefore the evidence for the observed results is strengthened.

Another strength of this thesis is the comparison of two different approaches regarding the metabolome dataset to strengthen the meaningfulness of the obtained results. Both approaches showed comparable and consisting results and strengthen the evidence for the observed associations.

To the authors' knowledge, the current thesis is the first approach of defining future weight-gaining phenotypes regarding subsequent body fat accumulation using an innovative average annual waist-hip difference. It was shown in chapter 4.2.2, that this difference is highly correlating with the WHR but with the clear advantage of better interpretability through its expression in absolute changes in cm/yr.

It must be emphasized that associations in the single metabolite approach are only present in for the WG phenotype in women when taking multiple testing into account. This may be due to several reasons. In order to create different phenotypes, the continuous variable 'waist-hip difference' was categorised. In analyses based on epidemiological data, conversion of continuous variables into categorical variables by grouping values is a common approach. But the simplicity achieved by creating artificial groups has a cost and may create problems. In particular, categorisation leads to a considerable loss of power and eventually loss of precision of estimated odds [151]. Second, fewer men than women were included in the analysis. It was not possible to identify predictive metabolites for the WG phenotype in men in the single metabolite approach. The fact of a decreased power due to categorisation of waist-hip difference as well as the lower number of men in the analytical

study population may have led to this negative result for men. Nevertheless, the principal component approach revealed a similar association in men compared to women, so that there might be a true association which could be identified with a greater power in the analysis for men.

As mentioned before, the analyses of objective 2 and 3 are based on the assumption that individual weight gain within the follow-up period appeared on the basis of a comparable metabolic profile as measured at baseline examination. There are differences in the reliability over time for the investigated metabolites. The assumption of time-stable metabolites is based on ICCs for p150 metabolites computed by Floegel *et al.* [108] during a 4-months period in fasting individuals. The follow-up periods in the present analysis are 7.1 years (KORA) and 8.6 years (EPIC-Potsdam) and it cannot be ruled out that metabolic changes appeared within this period. Thus, individual weight gain may have appeared on an (moderate) altered metabolic profile. It is unknown whether potential changes might affect the observed associations. This needs to be investigated in further studies.

Two metabolites (PC aa C38:1, PC ae C38:2) that showed a significant association with the WG phenotype in women had a poor ICC of less than 0.4, all other associated metabolites had fair to excellent ICC with the majority of 16 metabolites having at least good reliability of more than 0.51 ICC (Table 18) [108]. Hankinson *et al.* [152] reported attenuating effects for identified associations with decreasing ICC where derived risk estimations are likely to be underestimated. Below an ICC of 0.65, attenuations are markedly, above this value the attenuations are reported to be moderate. So the observed associations in of metabolites with an ICC of less than 0.65 may be attenuated and true associations may be even stronger. On the other hand, a low ICC may be an index of a lower biological relevance. Especially in terms of long-term changes of body composition, the impact of those metabolites may be lower compared to those metabolites with a high ICC that are reliable over time.

Table 18 Intraclass correlation coefficients of metabolites showing a significant association with the WG phenotype in women after correction for multiple testing (modified from Floegel *et al.* (2011) [108])

Metabolite	ICC	Metabolite	ICC
Amino acids		**Acyl-alkyl-PCs**	
Trp	0.45	PC ae C32:2	0.7
Lyso-PCs		PC ae C34:0	0.57
lysoPC a C17:0	0.64	PC ae C36:0	0.57
Diacyl-PCs		PC ae C36:1	0.65
PC aa C32:3	0.65	PC ae C36:2	0.76
PC aa C36:0	0.56	PC ae C38:0	0.61
PC aa C38:0	0.67	PC ae C38:2	0.29
PC aa C38:1	0.03	PC ae C40:1	0.59
PC aa C42:2	0.4	PC ae C40:2	0.72
PC aa C42:5	0.42	PC ae C40:5	0.6
		PC ae C40:6	0.69
		PC ae C42:2	0.56
		PC ae C42:3	0.59

Abbreviations: ICC, intraclass correlation coefficient; Trp, tryptophan; PC, phosphatidylcholine; a, acyl; e, alkyl

Most studies dealing with targeted metabolomics that have been considered in the discussion were cross-sectional whereas information from longitudinal studies is scarce. The results of this thesis are based on longitudinal data and cross-sectional associations may not give sufficient explanations for the observed results. Nevertheless, the considered studies in the discussion section give a consistent overview about potentially involved mechanisms, especially regarding the association to favourable lifestyle characteristics like physical activity.

Last limitation to mention is that not all participants provided their blood sample in a fasting state; nevertheless about 30% in EPIC-Potsdam and 88% KORA were fasting. To address this potential bias, a sensitivity analysis in a fasting population in EPIC-Potsdam was performed showing that the results were independent of fasting status.

5.4. Conclusion and Future Prospects

The majority of weight-gaining individuals in Germany tends to accumulate adipose tissue in the abdominal area. The results of this thesis support the necessity of primary prevention in terms of avoiding weight gain due to the increased disease risk with increasing abdominal adipose tissue.

The identification of metabolites from a targeted metabolomics approach revealed metabolic markers that showed inverse associations for the preferred deposition of body fat in the abdominal area. The aromatic AA tryptophan and in particular groups of PCs (diacyl, acyl-alkyl and lyso) were associated with a reduced chance of belonging to the WG phenotype. The identified metabolites may act as a marker of physiological processes that lead to the deposition of adipocytes in different areas of the human body. The underlying effects may represent a healthy lifestyle and therefore a favourable metabolic state. The aforementioned factors indicating a better health status (non-obese, insulin-sensitive, normotensive, higher CRF) were all shown to be inversely associated with chronic disease risk and should have synergetic effects with regard to prevention.

Future Prospects

With regard to elucidating the role of the identified metabolites in terms of primary prevention and common pathophysiological pathways, further prospective studies are needed to investigate especially metabolites of the phospholipid-metabolism as determinants for future body fat distribution. Despite this work was not able to link sexual hormones to metabolic profiles that were associated with one of the defined phenotypes, there is evidence that especially oestrogen and testosterone are involved in the deposition of body fat in specific areas of the human body and the underlying effects may include alterations in sexual hormones. However, future studies should investigate age-dependent changes of associated metabolites as well as dependencies to changes in sexual hormones.

In future studies, it would be interesting to investigate and possibly replicate the results obtained in this thesis in multi-level-analyses. Further analyses with summarized metabolite subclasses or metabolic pathways as a new level in multi-level-analysis would be interesting concerning the effects of clusters and/or groups of metabolites. Metabolic profiles are very complex and metabolites are exerting impact on each other and corresponding pathways. The application of PCA in this thesis was a first approach to cover the point of a more compact view on the metabolomics data and proposed multi-level-analysis would give an additional benefit in future studies when dealing with this kind of data.

Summary

Overweight and obesity are major public health problems in Germany. The associated comorbidities are highly depending on individual body fat distribution. A distinction is made between android and gynoid body fat distribution, which is highly depending on factors like age and sex. The present thesis aims to investigate general tendencies to one of these distributions using data from 4,126 weight-gaining individuals of three prospective German cohort studies. Furthermore, metabolic determinants for these phenotypes of body fat distribution are investigated based on targeted metabolomics data assessed with the Biocrates Absolute*IDQ*™ kits. The difference of average annual changes in waist and hip circumference was calculated to assess the tendency to android or gynoid body fat distribution. Sex-specific extrema-deciles of these differences were used to define waist-gaining and hip-gaining phenotypes, all remaining individuals formed the reference category. Multiple sex-specific logistic regression models adjusted for potential confounders with standardized metabolite concentration as independent and weight-gaining phenotype as dependent variable were fitted. Besides the single metabolite approach, a principal component analysis as a data-reduction method based on quality-controlled metabolite data was performed; corresponding principal components were as well used as independent variables in the pre-described logistic regression model. Overall, a general tendency to increase rather waist than hip circumference could be observed in men as well as in women. The identification of predictive metabolites showed tryptophan, diacyl-phosphatidylcholines C32:2, C36:0, C38:0, C38:1, C42:2, C42:5, acyl-alkyl-phosphatidylcholines C32:2, C34:0, C36:0, C36:1, C36:2, C38:0, C38:2, C40:1, C40:2, C40:5, C40:6, C42:2, C42:3 and lyso-phosphatidylcholine C17:0 to be inversely associated with the waist-gaining phenotype in women. For the waist-gaining phenotype in men and the hip-gaining phenotype in both sexes, no metabolite could be identified to be statistically significantly associated. The principal component analysis revealed biologically plausible principal components, of which one principal component mainly consisting of phosphatidylcholines was inversely associated with the waist-gaining phenotype in both sexes. Metabolites that have been identified to be inversely associated with the waist-gaining phenotype in women before all showed high factor loadings on the associated principal component. The identified or high-loading metabolites were all shown as being associated with favourable health conditions like increased fat free mass index, higher cardiorespiratory fitness, normotensive blood pressure and increased insulin-sensitivity. Weight-gaining individuals in Germany tend to increase abdominal body fat and especially the group of phosphatidylcholines was observed to be involved. Thus, future studies should investigate their role in future body fat deposition to increase our knowledge of associated pathophysiological pathways.

Zusammenfassung

Übergewicht und Adipositas stellen in Deutschland ein großes Problem dar. Die hiermit assoziierten Komorbiditäten sind in starker Weise abhängig von der zugrundeliegenden Körperfettverteilung. Hierbei werden die androide und die gynoide Körperform unterschieden. Die Verteilung des Körperfetts hängt stark von Faktoren wie Alter und Geschlecht ab. In der vorliegenden Arbeit sollte anhand der Daten dreier prospektiver Deutscher Kohortenstudien die generelle Tendenz von 4,126 gewichtszunehmenden Studienteilnehmern im Hinblick auf eine der beiden Verteilungsformen untersucht werden. Des Weiteren wurde auf Basis der Daten gerichteter Metabolom-Messungen mittels Biocrates Absolute*IDQ*™ Kits nach prädiktiven Metaboliten in gesucht, welche die Entstehung einer dieser beiden Phänotypen begünstigen. Für die Tendenz zu einer der beiden Verteilungsformen von Körperfett wurde die Differenz der durchschnittlichen Veränderungen von Taillen- und Hüftzunahme berechnet. Die geschlechts-spezifischen Extrem-Dezile dieser Differenz wurden als Taillen- bzw. Hüftzunehmer-Phänotyp definiert, die verbliebenen Individuen bildeten die Referenzkategorie. Für die Identifikation dieser prädiktiven Metabolite wurden multiple logistische Regressionsmodelle mit standardisierten Metabolit-Konzentrationen als unabhängige und den Phänotypen als abhängige Variable berechnet. Neben den Einzelmetaboliten wurde auch eine Hauptkomponentenanalyse auf Basis qualitätskontrollierter Metabolite durchgeführt, die Hauptkomponenten gingen ebenfalls als unabhängige Variablen in die logistische Regression ein. Als Ergebnis konnte eine eindeutige Tendenz zu einer vermehrten Zunahme an Taillenumfang im Vergleich zu Hüftumfang in beiden Geschlechtern beobachtet werden. Die Identifikation von prädiktiven Metaboliten bei Frauen lieferte Tryptophan, diacyl-Phosphatidylcholine C32:3, C36:0, C38:0, C38:1, C42:2, C42:5, acyl-alkyl-Phosphatidylcholine C32:2, C34:0, C36:0, C36:1, C36:2, C38:0, C38:2, C40:1, C40:2, C40:5, C40:6, C42:2, C42:3 und lyso-Phosphatidylcholin C17:0 als invers mit dem Taillenzunehmer-Phänotyp assoziiert. Für den Taillenzunehmer-Phänotyp bei Männern bzw. die Hüftzunehmer-Phänotyp bei beiden Geschlechtern konnten keine prädiktiven Metabolite identifiziert werden. Die Hauptkomponentenanalyse lieferte biologisch plausible Hauptkomponenten, von welchen eine aus Phosphatidylcholinen bestehende Komponente inverse Assoziation zum Taillenzunehmer-Phänotyp in beiden Geschlechtern zeigte. Die zuvor als invers bei Frauen assoziierten Metaboliten zeigten hohe Faktorladungen für diese Hauptkomponente und stehen in Verbindung mit gesundheitsrelevanten Faktoren wie erhöhter fettfreier Masse Index, höherer cardiorespiratorischer Fitness, normotensivem Blutdruck und erhöhter Insulinsensitivität. Gewichtszunehmende Individuen in Deutschland nehmen vermehrt im abdominellen Bereich zu. Insbesondere die Gruppe der Phosphatidylcholine scheint hier eine Rolle zu spielen und sollte hinsichtlich zukünftiger Körperfettanlagerung weiter untersucht werden, um pathophysiologische Zusammenhänge besser zu verstehen und aufzuklären.

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Appendix

Appendix 1 Impact of weighting for drop-out on average annual weight-hip-difference* in EPIC-Potsdam and DEGS

	not weighted for drop-out			weighted for drop-out		
	waist-hip difference (95% CI)	cut-offs p10	p90	waist-hip difference (95% CI)	cut-offs p10	p90
EPIC-Potsdam						
men	0.729 (0.682-0.776)	0.012	1.478	0.730 (0.683-0.777)	0.012	1.478
women	0.598 (0.561-0.635)	-0.057	1.243	0.597 (0.560-0.634)	-0.057	1.243
DEGS						
men	0.618 (0.586-0.650)	0.088	1.200	0.635 (0.603-0 668)	0.088	1.200
women	0.466 (0.430-0.502)	-0.213	1.120	0.449 (0.413-0.486)	-0.213	1.120

* (cm/yr)

Appendix 2 Pearson correlations among quality-controlled diacyl- and acyl-alkyl PCs for men and women in EPIC-Potsdam (PCs are ordered by type of bond and length of side chains as shown in Table 6 and Table 9)

Appendix 3 Results of sensitivity analyses in women for metabolites showing significant associations with WG phenotype in women

Metabolite	Study	Overall population OR (95% CI)	"Healthy" population OR (95% CI)	Non-menopausal population OR (95% CI)	Fasting population OR (95% CI)
Tryptophan	EPIC-Potsdam	0.67 (0.52-0.85)	0.70 (0.54-0.90)	0.70 (0.54-0.90)	1.14 (0.61-2.12)
	KORA	0.88 (0.56-1.36)	1.00 (0.63-1.59)	-	-
PC aa C32:3	EPIC-Potsdam	0.69 (0.54-0.88)	0.77 (0.60-1.00)	0.77 (0.60-1.00)	0.46 (0.24-0.89)
	KORA	0.69 (0.43-1.11)	0.73 (0.44-1.23)	-	-
PC aa C36:0	EPIC-Potsdam	0.69 (0.54-0.89)	0.80 (0.62-1.04)	0.80 (0.62-1.04)	0.67 (0.35-1.29)
	KORA	0.74 (0.47-1.16)	0.78 (0.49-1.24)	-	-
PC aa C38:0	EPIC-Potsdam	0.67 (0.52-0.86)	0.71 (0.55-0.94)	0.71 (0.55-0.94)	0.66 (0.35-1.25)
	KORA	0.78 (0.50-1.22)	0.88 (0.55-1.40)	-	-
PC aa C38:1	EPIC-Potsdam	0.73 (0.56-0.95)	0.69 (0.52-0.91)	0.69 (0.52-0.91)	0.79 (0.42-1.47)
	KORA	0.59 (0.37-0.95)	0.57 (0.35-0.95)	-	
PC aa C42:2	EPIC-Potsdam	0.64 (0.49-0.85)	0.67 (0.50-0.89)	0.67 (0.50-0.89)	0.65 (0.33-1.28)
	KORA	0.97 (0.61-1.54)	1.12 (0.70-1.80)	-	-
PC aa C42:5	EPIC-Potsdam	0.75 (0.58-0.99)	0.82 (0.62-1.08)	0.82 (0.62-1.08)	0.64 (0.31-1.34)
	KORA	0.62 (0.38-0.99)	0.54 (0.32-0.91)	-	-
PC ae C32:2	EPIC-Potsdam	0.72 (0.57-0.92)	0.80 (0.62-1.03)	0.80 (0.62-1.03)	0.62 (0.34-1.15)
	KORA	0.74 (0.48-1.15)	0.79 (0.49-1.25)	-	-
PC ae C34:0	EPIC-Potsdam	0.73 (0.57-0.94)	0.83 (0.65-1.08)	0.83 (0.65-1.08)	0.75 (0.41-1.38)
	KORA	0.70 (0.45-1.10)	0.71 (0.43-1.18)	-	-
PC ae C36:0	EPIC-Potsdam	0.71 (0.55-0.91)	0.69 (0.53-0.91)	0.69 (0.53-0.91)	0.65 (0.30-1.44)
	KORA	0.79 (0.48-1.29)	0.8 (0.47-1.36)	-	-

Table continued on the next page

Metabolite	Study	Overall population OR (95% CI)	"Healthy" population OR (95% CI)	Non-menopausal population OR (95% CI)	Fasting population OR (95% CI)
PC ae C36:1	EPIC-Potsdam	0.80 (0.63-1.03)	0.89 (0.69-1.15)	0.89 (0.69-1.15)	0.84 (0.44-1.60)
	KORA	0.52 (0.32-0.84)	0.49 (0.29-0.85)	-	-
PC ae C36:2	EPIC-Potsdam	0.77 (0.60-0.99)	0.88 (0.68-1.15)	0.88 (0.68-1.15)	0.59 (0.31-1.10)
	KORA	0.49 (0.30-0.82)	0.51 (0.30-0.88)	-	-
PC ae C38:0	EPIC-Potsdam	0.69 (0.54-0.90)	0.78 (0.59-1.02)	0.78 (0.59-1.02)	0.68 (0.34-1.37)
	KORA	0.64 (0.40-1.02)	0.64 (0.39-1.05)	-	-
PC ae C38:2	EPIC-Potsdam	0.77 (0.61-0.99)	0.81 (0.63-1.05)	0.81 (0.63-1.05)	0.57 (0.30-1.11)
	KORA	0.67 (0.43-1.05)	0.67 (0.41-1.08)	-	-
PC ae C40:1	EPIC-Potsdam	0.71 (0.55-0.90)	0.76 (0.59-0.99)	0.76 (0.59-0.99)	0.69 (0.36-1.33)
	KORA	0.67 (0.42-1.05)	0.72 (0.45-1.17)	-	
PC ae C40:2	EPIC-Potsdam	0.78 (0.60-1.00)	0.89 (0.68-1.15)	0.89 (0.68-1.15)	0.99 (0.53-1.85)
	KORA	0.81 (0.51-1.28)	0.6 (0.36-1.00)	-	-
PC ae C40:5	EPIC-Potsdam	0.71 (0.55-0.91)	0.77 (0.59-1.00)	0.77 (0.59-1.00)	0.88 (0.46-1.69)
	KORA	0.59 (0.37-0.93)	1.03 (0.63-1.70)	-	-
PC ae C40:6	EPIC-Potsdam	0.65 (0.50-0.84)	0.71 (0.54-0.94)	0.71 (0.54-0.94)	0.73 (0.42-1.30)
	KORA	0.67 (0.43-1.05)	0.75 (0.48-1.19)	-	-
PC ae C42:2	EPIC-Potsdam	0.62 (0.48-0.81)	0.63 (0.48-0.83)	0.63 (0.48-0.83)	0.69 (0.38-1.26)
	KORA	0.82 (0.55-1.22)	0.9 (0.59-1.38)	-	-
PC ae C42:3	EPIC-Potsdam	0.67 (0.52-0.86)	0.64 (0.49-0.84)	0.64 (0.49-0.84)	0.66 (0.37-1.18)
	KORA	0.67 (0.41-1.07)	0.73 (0.44-1.19)	-	-
lysoPC a C17:0	EPIC-Potsdam	0.70 (0.54-0.90)	0.72 (0.55-0.94)	0.72 (0.55-0.94)	0.80 (0.39-1.62)
	KORA	0.82 (0.53-1.27)	0.85 (0.54-1.35)	-	-

Abbreviations: PC, phosphatidylcholine; a, acyl; e, alkyl

Appendix 4 Results of subgroup analyses in women for metabolites showing significant associations with WG phenotype in women

Metabolite	Study	Overall study population OR (95% CI)	High physical activity ≥1h/week OR (95% CI)	Low physical activity <1h/week OR (95% CI)	Abdominal obesity (WC ≥ 88m) OR (95% CI)	No abdominal obesity (WC <88cm) OR (95% CI)	Age ≤ 55 years OR (95% CI)	Age > 55 years OR (95% CI)	Low alcohol consumption (<10g/d) OR (95% CI)	High alcohol consumption (≥10g/d) OR (95% CI)
Tryptophan	EPIC-Potsdam	0.67 (0.52-0.85)	0.69 (0.48-0.99)	0.71 (0.51-0.99)	1.22 (0.56-2.67)	0.67 (0.52-0.86)	0.74 (0.56-0.98)	0.61 (0.35-1.05)	0.67 (0.51-0.88)	0.74 (0.42-1.31)
	KORA	0.88 (0.56-1.36)	1.03 (0.53-2.01)	0.78 (0.43-1.43)	0.62 (0.30-1.32)	1.12 (0.63-1.99)	-	-	0.72 (0.43-1.20)	3.7 (0.76-18.09)
PC aa C32:3	EPIC-Potsdam	0.69 (0.54-0.88)	0.89 (0.63-1.27)	0.61 (0.44-0.86)	0.80 (0.35-1.85)	0.72 (0.56-0.93)	0.72 (0.53-0.96)	0.78 (0.47-1.28)	0.73 (0.56-0.95)	0.69 (0.35-1.34)
	KORA	0.69 (0.43-1.11)	0.81 (0.46-1.43)	0.38 (0.17-0.89)	0.99 (0.50-1.95)	0.23 (0.08-0.62)	-	-	0.49 (0.27-0.91)	0.66 (0.28-1.56)
PC aa C36:0	EPIC-Potsdam	0.69 (0.54-0.89)	0.80 (0.56-1.16)	0.65 (0.47-0.92)	0.27 (0.08-0.98)	0.77 (0.60-1.00)	0.74 (0.54-1.00)	0.68 (0.43-1.08)	0.70 (0.53-0.92)	0.87 (0.50-1.53)
	KORA	0.74 (0.47-1.16)	0.82 (0.42-1.63)	0.63 (0.33-1.21)	0.71 (0.30-1.71)	0.8 (0.47-1.36)	-	-	0.55 (0.31-0.98)	3.19 (0.85-12.03)
PC aa C38:0	EPIC-Potsdam	0.67 (0.52-0.86)	0.69 (0.47-1.00)	0.69 (0.49-0.95)	0.27 (0.08-0.93)	0.72 (0.56-0.93)	0.67 (0.49-0.91)	0.72 (0.47-1.10)	0.66 (0.50-0.86)	0.87 (0.49-1.55)
	KORA	0.78 (0.50-1.22)	0.8 (0.40-1.58)	0.72 (0.38-1.37)	0.76 (0.32-1.79)	0.86 (0.50-1.46)	-	-	0.71 (0.42-1.2)	2.47 (0.57-10.76)
PC aa C38:1	EPIC-Potsdam	0.73 (0.56-0.95)	0.63 (0.42-0.94)	0.68 (0.48-0.98)	0.62 (0.22-1.70)	0.68 (0.52-0.90)	0.62 (0.44-0.87)	0.77 (0.50-1.20)	0.64 (0.48-0.86)	0.87 (0.51-1.49)
	KORA	0.59 (0.37-0.95)	0.85 (0.42-1.70)	0.36 (0.17-0.75)	0.53 (0.22-1.25)	0.62 (0.35-1.12)	-	-	0.42 (0.23-0.76)	2.4 (0.84-6.87)

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Metabolite	Study	Overall study population OR (95% CI)	High physical activity ≥1h/week OR (95% CI)	Low physical activity <1h/week OR (95% CI)	Abdominal obesity (WC ≥ 88m) OR (95% CI)	No abdominal obesity (WC <88cm) OR (95% CI)	Age ≤ 55 years OR (95% CI)	Age > 55 years OR (95% CI)	Low alcohol consumption (<10g/d) OR (95% CI)	High alcohol consumption (≥10g/d) OR (95% CI)
PC aa C42:2	EPIC-Potsdam	0.64 (0.49-0.85)	0.64 (0.43-0.96)	0.58 (0.39-0.85)	0.33 (0.10-1.12)	0.66 (0.50-0.87)	0.57 (0.40-0.81)	0.74 (0.48-1.15)	0.57 (0.42-0.78)	0.91 (0.51-1.62)
	KORA	0.97 (0.61-1.54)	1.3 (0.68-2.47)	0.66 (0.32-1.35)	1.26 (0.57-2.82)	0.84 (0.47-1.51)	-	-	0.79 (0.45-1.39)	3.01 (0.86-10.51)
PC aa C42:5	EPIC-Potsdam	0.75 (0.58-0.99)	0.81 (0.56-1.18)	0.66 (0.45-0.96)	0.49 (0.13-1.83)	0.76 (0.58-0.99)	0.77 (0.55-1.06)	0.69 (0.43-1.09)	0.71 (0.53-0.96)	0.88 (0.47-1.64)
	KORA	0.62 (0.38-0.99)	0.49 (0.21-1.12)	0.64 (0.35-1.18)	0.54 (0.21-1.37)	0.6 (0.33-1.10)	-	-	0.57 (0.32-1.00)	0.5 (0.15-1.66)
PC ae C32:2	EPIC-Potsdam	0.72 (0.57-0.92)	0.69 (0.47-1.00)	0.82 (0.60-1.10)	0.82 (0.28-2.37)	0.76 (0.59-0.97)	0.72 (0.54-0.97)	0.93 (0.60-1.43)	0.69 (0.53-0.90)	1.17 (0.73-1.89)
	KORA	0.74 (0.48-1.15)	0.69 (0.33-1.45)	0.7 (0.40-1.23)	0.95 (0.45-2.00)	0.62 (0.35-1.11)	-	-	0.67 (0.40-1.13)	1.2 (0.46-3.12)
PC ae C34:0	EPIC-Potsdam	0.73 (0.57-0.94)	0.67 (0.48-0.95)	0.83 (0.33-2.11)	0.78 (0.61-1.01)	0.76 (0.56-1.03)	0.87 (0.55-1.38)	0.87 (0.55-1.38)	0.79 (0.61-1.02)	0.76 (0.39-1.48)
	KORA	0.70 (0.45-1.10)	0.75 (0.34-1.66)	0.7 (0.40-1.21)	1.07 (0.53-2.15)	0.5 (0.26-0.96)	-	-	0.65 (0.39-1.09)	1.12 (0.40-3.13)
PC ae C36:0	EPIC-Potsdam	0.71 (0.55-0.91)	0.75 (0.53-1.05)	0.69 (0.48-0.98)	0.06 (0.01-0.56)	0.78 (0.61-1.01)	0.74 (0.54-1.00)	0.72 (0.46-1.14)	0.64 (0.48-0.86)	1.10 (0.65-1.88)
	KORA	0.79 (0.48-1.29)	0.83 (0.40-1.74)	0.7 (0.35-1.42)	1.18 (0.55-2.53)	0.59 (0.28-1.22)	-	-	0.73 (0.42-1.27)	1.48 (0.35-6.19)
PC ae C36:1	EPIC-Potsdam	0.80 (0.63-1.03)	1.08 (0.74-1.57)	0.66 (0.47-0.92)	1.73 (0.66-4.58)	0.77 (0.60-0.99)	0.71 (0.52-0.97)	1.08 (0.68-1.71)	0.82 (0.63-1.06)	0.83 (0.44-1.57)
	KORA	0.52 (0.32-0.84)	0.54 (0.24-1.20)	0.52 (0.28-0.98)	0.84 (0.36-1.99)	0.37 (0.18-0.76)	-	-	0.55 (0.32-0.95)	0.65 (0.15-2.81)
PC ae C36:2	EPIC-Potsdam	0.77 (0.60-0.99)	0.88 (0.60-1.30)	0.73 (0.52-1.02)	2.37 (0.73-7.74)	0.78 (0.60-1.01)	0.74 (0.54-1.01)	1.07 (0.66-1.71)	0.79 (0.60-1.04)	0.83 (0.44-1.58)
	KORA	0.49	0.62	0.42	1.03	0.31	-	-	0.53	0.78

Table continued on the next page

Metabolite	Study	Overall study population OR (95% CI)	High physical activity ≥1h/week OR (95% CI)	Low physical activity <1h/week OR (95% CI)	Abdominal obesity (WC ≥ 88m) OR (95% CI)	No abdominal obesity (WC <88cm) OR (95% CI)	Age ≤ 55 years OR (95% CI)	Age > 55 years OR (95% CI)	Low alcohol consumption (<10g/d) OR (95% CI)	High alcohol consumption (≥10g/d) OR (95% CI)
		(0.30-0.82)	(0.28-1.35)	(0.21-0.84)	(0.41-2.59)	(0.15-0.65)			(0.30-0.93)	(0.21-2.93)
PC ae C38:0	EPIC-Potsdam	0.69 (0.54-0.90)	0.85 (0.60-1.21)	0.56 (0.38-0.82)	0.49 (0.16-1.46)	0.73 (0.57-0.95)	0.73 (0.53-1.00)	0.71 (0.46-1.10)	0.73 (0.56-0.96)	0.72 (0.38-1.38)
	KORA	0.64 (0.40-1.02)	0.82 (0.43-1.58)	0.49 (0.24-1.00)	0.55 (0.24-1.29)	0.69 (0.39-1.22)	-	-	0.45 (0.25-0.81)	1.67 (0.57-4.88)
PC ae C38:2	EPIC-Potsdam	0.77 (0.61-0.99)	0.89 (0.63-1.25)	0.68 (0.48-0.97)	1.44 (0.49-4.22)	0.79 (0.62-1.01)	0.73 (0.54-0.98)	1.08 (0.69-1.67)	0.74 (0.57-0.97)	1.08 (0.61-1.90)
	KORA	0.67 (0.43-1.05)	0.75 (0.38-1.49)	0.59 (0.32-1.09)	1.63 (0.74-3.60)	0.4 (0.21-0.78)	-	-	0.69 (0.42-1.12)	1.87 (0.54-6.46)
PC ae C40:1	EPIC-Potsdam	0.71 (0.55-0.90)	0.85 (0.60-1.20)	0.62 (0.43-0.88)	0.60 (0.21-1.71)	0.75 (0.59-0.97)	0.70 (0.52-0.95)	0.85 (0.54-1.34)	0.73 (0.56-0.95)	0.85 (0.48-1.52)
	KORA	0.67 (0.42-1.05)	0.84 (0.42-1.66)	0.54 (0.28-1.07)	0.52 (0.23-1.22)	0.76 (0.44-1.31)	-	-	0.5 (0.29-0.88)	2.34 (0.72-7.65)
PC ae C40:2	EPIC-Potsdam	0.78 (0.60-1.0)	0.88 (0.59-1.30)	0.80 (0.57-1.12)	1.23 (0.59-2.58)	0.77 (0.59-1.01)	0.86 (0.63-1.16)	0.82 (0.51-1.31)	0.81 (0.62-1.06)	0.94 (0.52-1.69)
	KORA	0.81 (0.51-1.28)	0.72 (0.34-1.52)	0.53 (0.30-0.97)	0.97 (0.42-2.25)	0.47 (0.26-0.87)	-	-	0.68 (0.41-1.13)	0.5 (0.11-2.13)
PC ae C40:5	EPIC-Potsdam	0.71 (0.55-0.91)	0.85 (0.57-1.27)	0.67 (0.48-0.93)	0.61 (0.18-2.03)	0.72 (0.56-0.94)	0.70 (0.52-0.95)	0.87 (0.54-1.43)	0.68 (0.52-0.90)	0.87 (0.47-1.60)
	KORA	0.59 (0.37-0.93)	1.11 (0.54-2.30)	0.68 (0.37-1.25)	0.82 (0.35-1.95)	0.8 (0.45-1.42)	-	-	0.83 (0.49-1.40)	1.49 (0.40-5.55)
PC ae C40:6	EPIC-Potsdam	0.65 (0.50-0.84)	0.72 (0.48-1.07)	0.63 (0.44-0.89)	0.60 (0.24-1.49)	0.67 (0.51-0.88)	0.67 (0.49-0.93)	0.68 (0.43-1.07)	0.68 (0.51-0.89)	0.68 (0.36-1.29)
	KORA	0.67 (0.43-1.05)	0.81 (0.42-1.58)	0.58 (0.31-1.10)	0.56 (0.22-1.44)	0.74 (0.44-1.25)	-	-	0.63 (0.38-1.06)	1.4 (0.43-4.56)

Table continued on the next page

Metabolite	Study	Overall study population OR (95% CI)	High physical activity ≥1h/week OR (95% CI)	Low physical activity <1h/week OR (95% CI)	Abdominal obesity (WC ≥ 88m) OR (95% CI)	No abdominal obesity (WC <88cm) OR (95% CI)	Age ≤ 55 years OR (95% CI)	Age > 55 years OR (95% CI)	Low alcohol consumption (<10g/d) OR (95% CI)	High alcohol consumption (≥10g/d) OR (95% CI)
PC ae C42:2	EPIC-Potsdam	0.62 (0.48-0.81)	0.68 (0.46-1.00)	0.55 (0.38-0.79)	0.40 (0.13-1.26)	0.62 (0.47-0.81)	0.56 (0.41-0.77)	0.72 (0.44-1.17)	0.61 (0.46-0.82)	0.53 (0.27-1.05)
	KORA	0.82 (0.55-1.22)	1.01 (0.52-1.96)	0.72 (0.42-1.23)	1.01 (0.55-1.86)	0.69 (0.40-1.18)	-	-	0.75 (0.47-1.20)	2.5 (0.58-10.69)
PC ae C42:3	EPIC-Potsdam	0.67 (0.52-0.86)	0.71 (0.49-1.03)	0.57 (0.39-0.81)	0.55 (0.17-1.77)	0.64 (0.49-0.83)	0.61 (0.45-0.82)	0.78 (0.48-1.27)	0.63 (0.48-0.83)	0.60 (0.32-1.14)
	KORA	0.67 (0.41-1.07)	0.81 (0.40-1.66)	0.56 (0.29-1.10)	0.83 (0.36-1.94)	0.57 (0.30-1.11)	-	-	0.55 (0.31-0.98)	3.77 (0.70-20.29)
lysoPC a C17:0	EPIC-Potsdam	0.70 (0.54-0.90)	0.74 (0.49-1.13)	0.70 (0.50-0.97)	2.19 (0.93-5.13)	0.64 (0.49-0.84)	0.60 (0.44-0.82)	1.08 (0.66-1.77)	0.74 (0.56-0.98)	0.58 (0.32-1.07)
	KORA	0.82 (0.53-1.27)	1.55 (0.89-2.72)	0.41 (0.19-0.89)	0.53 (0.18-1.61)	0.91 (0.56-1.48)	-	-	0.86 (0.53-1.40)	0.84 (0.19-3.73)

Abbreviations: PC, phosphatidylcholine; a, acyl; e, alkyl

Appendix 5 Values of I^2 as an index of present heterogeneity in the fixed-effects meta-analyses for investigated endpoints in men and women

	Men		Women	
	WG	**HG**	**WG**	**HG**
	I^2	I^2	I^2	I^2
Amino Acids				
Arginine	57%	0%	0%	26%
Glutamine	62%	0%	43%	0%
Glycine	0%	77%	72%	0%
Histidine	73%	0%	0%	0%
Methionine	65%	0%	71%	0%
Ornithine	85%	0%	3%	0%
Phenylalanine	0%	72%	0%	27%
Proline	0%	0%	0%	0%
Serine	69%	0%	77%	40%
Threonine	0%	0%	77%	61%
Tryptophan	55%	0%	12%	0%
Tyrosine	59%	0%	0%	0%
Valine	61%	59%	25%	17%
Hexose				
H1	0%	0%	11%	0%
Acylcarnitines				
C0	0%	0%	0%	0%
C2	0%	0%	0%	0%
C3	0%	55%	0%	0%
C5-OH (C3-DC-M)	0%	0%	0%	0%
C7-DC	0%	0%	3%	70%
C9	0%	0%	0%	0%
C10	0%	0%	0%	0%
C10:2	0%	8%	0%	42%
C14:1	0%	0%	0%	0%
C14:2	0%	34%	0%	0%
C16	86%	0%	0%	0%
C18	10%	52%	0%	0%
C18:1	64%	0%	0%	0%
C18:2	63%	3%	0%	24%
Diacyl-Phosphatidylcholines				
PC aa C28:1	0%	75%	87%	0%
PC aa C30:0	0%	68%	0%	0%
PC aa C32:0	54%	0%	0%	45%
PC aa C32:1	71%	0%	0%	0%
PC aa C32:2	0%	60%	56%	0%
PC aa C32:3	81%	0%	0%	44%
PC aa C34:1	79%	0%	0%	0%
PC aa C34:2	75%	0%	40%	0%

Table continued on the next page

	Men		Women	
	WG	HG	WG	HG
	I^2	I^2	I^2	I^2
PC aa C34:3	84%	0%	0%	0%
PC aa C34:4	30%	59%	64%	9%
PC aa C36:0	63%	0%	0%	82%
PC aa C36:1	0%	0%	0%	0%
PC aa C36:2	7%	0%	12%	0%
PC aa C36:3	59%	0%	75%	0%
PC aa C36:4	52%	0%	70%	0%
PC aa C36:5	0%	40%	0%	38%
PC aa C36:6	37%	63%	0%	0%
PC aa C38:0	65%	0%	0%	84%
PC aa C38:1	64%	0%	0%	0%
PC aa C38:3	0%	0%	87%	0%
PC aa C38:4	0%	0%	72%	0%
PC aa C38:5	23%	0%	0%	0%
PC aa C38:6	71%	0%	26%	65%
PC aa C40:2	0%	0%	0%	0%
PC aa C40:3	18%	51%	0%	3%
PC aa C40:4	2%	0%	50%	0%
PC aa C40:5	52%	0%	22%	0%
PC aa C40:6	58%	0%	0%	47%
PC aa C42:0	50%	0%	56%	46%
PC aa C42:1	79%	21%	44%	23%
PC aa C42:2	78%	0%	56%	82%
PC aa C42:4	60%	0%	0%	60%
PC aa C42:5	61%	0%	0%	0%
PC aa C42:6	79%	0%	0%	0%
Acyl-alkyl-Phosphatidylcholines				
PC ae C30:0	20%	71%	0%	0%
PC ae C30:2	0%	0%	74%	0%
PC ae C32:1	69%	0%	0%	75%
PC ae C32:2	42%	0%	0%	85%
PC ae C34:0	0%	61%	0%	0%
PC ae C34:1	63%	24%	0%	43%
PC ae C34:2	0%	28%	36%	89%
PC ae C34:3	0%	0%	0%	91%
PC ae C36:0	39%	0%	0%	0%
PC ae C36:1	36%	65%	59%	0%
PC ae C36:2	30%	0%	58%	27%
PC ae C36:3	0%	0%	4%	86%
PC ae C36:4	0%	0%	0%	3%
PC ae C36:5	0%	0%	0%	65%
PC ae C38:0	68%	0%	0%	71%
PC ae C38:1	0%	0%	0%	0%

Table continued on the next page

	Men		Women	
	WG	HG	WG	HG
	I^2	I^2	I^2	I^2
PC ae C38:2	77%	0%	0%	0%
PC ae C38:3	2%	64%	84%	0%
PC ae C38:4	0%	0%	21%	34%
PC ae C38:5	0%	0%	0%	71%
PC ae C38:6	0%	0%	0%	80%
PC ae C40:1	67%	0%	0%	52%
PC ae C40:2	0%	0%	6%	52%
PC ae C40:3	32%	36%	21%	62%
PC ae C40:4	46%	0%	0%	12%
PC ae C40:5	47%	0%	0%	64%
PC ae C40:6	77%	0%	0%	84%
PC ae C42:1	14%	0%	0%	0%
PC ae C42:2	61%	0%	20%	35%
PC ae C42:3	63%	0%	0%	81%
PC ae C42:4	0%	0%	0%	65%
PC ae C42:5	57%	0%	0%	33%
PC ae C44:3	82%	65%	0%	0%
PC ae C44:4	0%	0%	0%	0%
PC ae C44:5	66%	0%	0%	7%
PC ae C44:6	77%	0%	41%	62%
Lyso-Phosphatidylcholines				
lysoPC a C14:0	0%	42%	0%	61%
lysoPC a C16:0	27%	0%	0%	0%
lysoPC a C16:1	70%	42%	0%	0%
lysoPC a C17:0	0%	25%	0%	0%
lysoPC a C18:0	0%	0%	0%	0%
lysoPC a C18:1	0%	0%	81%	0%
lysoPC a C18:2	0%	0%	66%	0%
lysoPC a C20:3	0%	64%	0%	0%
lysoPC a C20:4	0%	0%	64%	0%
lysoPC a C28:1	0%	0%	3%	0%
Sphingomyelins				
SM C16:0	33%	0%	0%	80%
SM C16:1	54%	0%	0%	81%
SM C18:0	0%	0%	79%	46%
SM C18:1	0%	0%	19%	65%
SM C20:2	11%	0%	0%	69%
SM C24:0	0%	56%	22%	0%
SM C24:1	0%	0%	0%	54%
SM C26:1	0%	0%	0%	73%
SM (OH) C14:1	0%	59%	83%	20%
SM (OH) C16:1	0%	0%	70%	38%
SM (OH) C22:1	0%	67%	67%	0%

Table continued on the next page

	Men		Women	
	WG	**HG**	**WG**	**HG**
	I^2	I^2	I^2	I^2
SM (OH) C22:2	0%	43%	76%	52%
SM (OH) C24:1	0%	79%	0%	0%

Abbreviations: PC, phosphatidylcholine; a, acyl; e, alkyl; SM, sphingomyelin

Appendix 6 Rotated Factor Pattern of 5 principal components in men

	Rotated factor pattern men				
	PComp1	PComp2	PComp3	PComp4	PComp5
Amino Acids					
Arginine	4	9	14	63*	5
Glutamine	-8	17	15	68*	4
Glycine	-11	21	5	55	-6
Histidine	14	9	7	76*	7
Methionine	11	2	-11	79*	-7
Ornithine	1	7	-4	70*	6
Phenylalanine	19	4	7	75*	10
Proline	11	2	0	51	-16
Serine	-8	26	8	63*	1
Threonine	11	15	-5	65*	-16
Tryptophan	14	13	10	77*	0
Tyrosine	20	-7	-4	67*	1
Valine	14	-2	3	72*	3
xLeucine	13	-4	0	77*	3
Hexose					
H1	23	4	1	26	8
Acylcarnitines					
C0	27	-5	-5	39	35
C2	24	7	1	-5	57
C3	14	-8	3	46	16
C5-DC (C6-OH)	2	4	17	3	63*
C5-OH (C3-DC-M)	9	-2	20	10	53
C7-DC	2	4	8	-10	69*
C8:1	-6	11	-11	15	49
C9	-5	12	31	-4	26
C10	6	9	11	-9	45
C10:2	3	5	12	5	54
C14:1	21	13	25	-7	70*
C14:2	0	11	0	-9	76*
C16	40	9	13	8	55
C16:2	4	13	2	-8	65*
C18	20	14	27	20	38
C18:1	28	10	-1	6	63*
C18:2	-2	15	-13	19	61*
Diacyl-Phosphatidylcholines					
PC aa C28:1	36	18	68*	2	4
PC aa C30:0	74*	6	33	-2	-8
PC aa C32:0	73*	27	27	9	8
PC aa C32:1	82*	-5	-8	-5	4
PC aa C32:2	75*	4	12	7	-17
PC aa C32:3	45	46	32	7	-12
PC aa C34:1	84*	10	11	7	13
PC aa C34:2	52	24	21	35	-11
PC aa C34:3	78*	19	7	11	-9
PC aa C34:4	83*	13	7	11	-5
PC aa C36:0	30	69*	16	10	7
PC aa C36:1	82*	12	21	9	12
PC aa C36:2	54	25	24	37	-6
PC aa C36:3	75*	12	22	28	0
PC aa C36:4	68*	17	11	27	17

	Rotated factor pattern men				
	PComp1	**PComp2**	**PComp3**	**PComp4**	**PComp5**
PC aa C36:5	53	45	-3	-12	14
PC aa C36:6	64*	50	2	-9	4
PC aa C38:0	13	80*	19	14	11
PC aa C38:1	14	57	6	-6	6
PC aa C38:3	71*	-3	20	19	22
PC aa C38:4	62*	11	9	24	29
PC aa C38:5	76*	38	6	6	23
PC aa C38:6	51	55	2	-4	23
PC aa C40:2	26	71*	-2	1	14
PC aa C40:3	27	68*	2	-1	15
PC aa C40:4	74*	0	7	18	26
PC aa C40:5	81*	12	2	10	27
PC aa C40:6	55	39	-1	-4	25
PC aa C42:0	-14	84*	17	7	9
PC aa C42:1	0	81*	12	13	15
PC aa C42:2	31	74*	4	0	15
PC aa C42:4	41	54	15	12	22
PC aa C42:5	52	49	-6	-2	21
PC aa C42:6	62*	48	-1	-6	18
Acyl-alkyl-Phosphatidylcholines					
PC ae C30:0	32	32	61*	4	-27
PC ae C30:1	16	17	34	-7	-6
PC ae C30:2	21	29	45	1	3
PC ae C32:1	37	53	43	12	-2
PC ae C32:2	41	62*	40	3	-2
PC ae C34:0	49	34	58	0	-20
PC ae C34:1	55	32	56	6	-15
PC ae C34:2	7	43	46	26	-25
PC ae C34:3	7	50	34	24	-7
PC ae C36:0	52	54	14	1	12
PC ae C36:1	47	29	64*	1	-13
PC ae C36:2	14	41	60	21	-32
PC ae C36:3	17	45	40	29	-16
PC ae C36:4	20	35	29	32	4
PC ae C36:5	32	52	25	22	15
PC ae C38:0	56	64*	6	-1	8
PC ae C38:1	31	40	41	1	5
PC ae C38:2	38	52	43	20	-18
PC ae C38:3	37	30	62*	17	-16
PC ae C38:4	18	39	54	34	-1
PC ae C38:5	21	53	29	30	13
PC ae C38:6	26	73*	23	12	13
PC ae C40:1	52	63*	12	19	6
PC ae C40:2	17	45	63*	-6	5
PC ae C40:3	19	58	59	12	0
PC ae C40:4	10	56	45	34	1
PC ae C40:5	16	73*	37	15	7
PC ae C40:6	12	77*	36	7	3
PC ae C42:1	53	42	10	15	24
PC ae C42:2	47	65*	26	2	10
PC ae C42:3	30	79*	22	11	7
PC ae C42:4	-12	57	39	26	4

	Rotated factor pattern men				
	PComp1	**PComp2**	**PComp3**	**PComp4**	**PComp5**
PC ae C42:5	-11	70*	31	22	8
PC ae C44:3	24	53	20	8	16
PC ae C44:4	-4	48	33	19	3
PC ae C44:5	-13	50	28	22	12
PC ae C44:6	-20	69*	19	22	11
Lyso-Phosphatidylcholines					
lysoPC a C14:0	48	-6	20	18	-5
lysoPC a C16:0	44	16	15	41	16
lysoPC a C16:1	75*	1	-7	13	10
lysoPC a C17:0	3	23	44	21	-19
lysoPC a C18:0	26	22	16	42	11
lysoPC a C18:1	44	19	9	29	4
lysoPC a C18:2	1	22	12	41	-13
lysoPC a C20:3	49	-3	9	35	3
lysoPC a C20:4	30	16	5	35	14
lysoPC a C28:1	22	18	48	4	-6
Sphingomyelins					
SM (OH) C14:1	0	18	85*	1	-1
SM (OH) C16:1	-7	14	89*	1	7
SM (OH) C22:1	8	8	84*	8	20
SM (OH) C22:2	-1	24	84*	4	15
SM (OH) C24:1	0	0	80*	3	25
SM C16:0	7	29	75*	14	32
SM C16:1	15	26	66*	13	38
SM C18:0	6	2	73*	4	38
SM C18:1	4	11	68*	9	34
SM C20:2	-8	38	15	-3	4
SM C24:0	20	3	62*	12	43
SM C24:1	4	30	53	7	40
SM C26:0	2	6	69*	-5	29
SM C26:1	7	22	51	-4	41

Factor loadings are multiplied by 100 and rounded.
Values greater 0.6 are marked with '*'
Abbreviations: PC, phosphatidylcholine; a, acyl; e, alkyl; SM, sphingomyelin

Appendix 7 Rotated Factor Pattern of 4 principal components in women

	Rotated factor pattern women			
	PComp1	**PComp2**	**PComp3**	**PComp4**
Amino Acids				
Arginine	15	1	14	66*
Glutamine	26	-1	11	66*
Glycine	23	-16	12	31
Histidine	18	15	-7	60*
Methionine	19	5	-16	75*
Ornithine	7	-5	16	68*
Phenylalanine	13	14	2	74*
Proline	0	3	-2	67*
Serine	41	-15	4	55
Threonine	28	11	-21	51
Tryptophan	27	16	-6	74*
Tyroysine	0	12	3	68*
Valine	-5	16	-2	70*
xLeucine	-2	10	-4	72*
Hexose				
H1	-4	24	12	27
Acylcarnitines				
C0	-6	13	29	43
C2	8	5	44	-19
C3	-13	12	23	54
C5-DC (C6-OH)	1	-2	54	11
C5-OH (C3-DC-M)	-9	3	51	20
C7-DC	-1	-12	61*	-1
C8:1	-4	-8	32	16
C9	3	3	45	17
C10	2	0	48	-6
C10:2	-6	-8	52	19
C14:1	8	13	70*	-8
C14:2	0	-15	64*	-11
C16	6	26	57	4
C16:2	-4	-12	56	4
C18	19	8	45	18
C18:1	11	5	53	-5
C18:2	10	-20	50	10
Diacyl-Phosphatidylcholines				
PC aa C28:1	25	48	50	15
PC aa C30:0	12	77*	7	12
PC aa C32:0	39	75*	5	6
PC aa C32:1	-7	80*	-8	6
PC aa C32:2	6	74*	-11	12
PC aa C32:3	37	59	15	20
PC aa C34:1	14	84*	-1	5
PC aa C34:2	34	60*	-14	15
PC aa C34:3	19	75*	-10	22
PC aa C34:4	9	85*	-12	6
PC aa C36:0	67*	28	9	0
PC aa C36:1	13	74*	21	22
PC aa C36:2	34	55	10	31
PC aa C36:3	18	78*	-3	22
PC aa C36:4	23	72*	-7	-2

PC aa C36:5	24	55	7	-10
PC aa C36:6	29	72*	2	-2
PC aa C38:0	76*	21	15	1
PC aa C38:1	50	1	10	-3
PC aa C38:3	-1	73*	28	16
PC aa C38:4	20	65*	22	4
PC aa C38:5	31	74*	21	2
PC aa C38:6	39	55	11	-13
PC aa C40:2	52	19	10	7
PC aa C40:3	55	26	14	9
PC aa C40:4	8	73*	23	13
PC aa C40:5	9	68*	31	10
PC aa C40:6	23	51	28	-3
PC aa C42:0	83*	-11	14	5
PC aa C42:1	81*	-3	14	9
PC aa C42:2	64*	19	14	5
PC aa C42:4	57	41	17	5
PC aa C42:5	44	48	7	-5
PC aa C42:6	40	56	17	3
Acyl-alkyl-Phosphatidylcholines				
PC ae C30:0	45	44	9	17
PC ae C30:1	26	11	20	3
PC ae C30:2	36	22	41	15
PC ae C32:1	71*	40	5	8
PC ae C32:2	71*	39	17	8
PC ae C34:0	40	65*	14	9
PC ae C34:1	46	66*	9	9
PC ae C34:2	57	33	-3	14
PC ae C34:3	68*	22	-3	9
PC ae C36:0	53	39	10	8
PC ae C36:1	34	63*	26	11
PC ae C36:2	54	39	10	23
PC ae C36:3	58	37	-2	18
PC ae C36:4	52	39	2	3
PC ae C36:5	63*	40	9	-3
PC ae C38:0	52	57	9	5
PC ae C38:1	36	37	30	9
PC ae C38:2	57	43	8	28
PC ae C38:3	37	61*	20	18
PC ae C38:4	63*	42	13	8
PC ae C38:5	68*	33	12	3
PC ae C38:6	69*	38	15	-4
PC ae C40:1	62*	50	7	20
PC ae C40:2	50	37	43	1
PC ae C40:3	71*	28	30	20
PC ae C40:4	78*	21	9	17
PC ae C40:5	82*	24	22	8
PC ae C40:6	77*	27	24	0
PC ae C42:1	48	44	19	18
PC ae C42:2	61*	38	27	18
PC ae C42:3	78*	19	14	20
PC ae C42:4	76*	-4	6	19
PC ae C42:5	85*	-5	8	10
PC ae C44:3	57	14	16	20

PC ae C44:4	63*	0	9	16
PC ae C44:5	73*	-7	8	9
PC ae C44:6	82*	-13	10	9
Lyso-Phosphatidylcholines				
lysoPC a C14:0	-11	46	6	37
lysoPC a C16:0	7	33	19	52
lysoPC a C16:1	-6	49	10	42
lysoPC a C17:0	23	12	28	40
lysoPC a C18:0	15	16	31	48
lysoPC a C18:1	20	17	10	51
lysoPC a C18:2	25	-10	-2	54
lysoPC a C20:3	-7	42	8	45
lysoPC a C20:4	20	20	10	34
lysoPC a C28:1	31	22	28	17
Sphingomyelins				
SM (OH) C14:1	32	22	62*	11
SM (OH) C16:1	32	17	69*	7
SM (OH) C22:1	21	36	67*	10
SM (OH) C22:2	35	26	68*	6
SM (OH) C24:1	18	19	71*	4
SM C16:0	45	19	67*	12
SM C16:1	30	28	69*	12
SM C18:0	18	23	73*	-1
SM C18:1	23	20	69*	4
SM C20:2	23	-1	23	12
SM C24:0	16	37	61*	7
SM C24:1	42	14	49	-3
SM C26:0	21	12	65*	1
SM C26:1	28	12	63*	-9

Factor loadings are multiplied by 100 and rounded.
Values greater 0.6 are marked with '*'
Abbreviations: PC, phosphatidylcholine; a, acyl; e, alkyl; SM, sphingomyelin

Appendix 8 Pearson correlation coefficients for Absolute IDQ p150 metabolites with sex hormones estradiol and testosterone stratified by sex in EPIC-Potsdam

	Men				Women			
	Estradiol (n=261)		Testosterone (n=317)		Estradiol (n=438)		Testosterone (n=189)	
	r	p	r	p	r	p	r	p
Amino Acids								
Arginine	-0.0002	0.9972	-0.0690	0.2209	0.0015	0.9759	0.0397	0.5879
Glutamine	-0.0705	0.2564	-0.0114	0.8394	0.0636	0.184	0.0478	0.5134
Glycine	0.0481	0.439	0.0088	0.8757	0.0029	0.9524	-0.0069	0.9249
Histidine	-0.0485	0.4349	-0.0960	0.088	-0.0135	0.7778	0.0966	0.1863
Methionine	-0.0682	0.2721	-0.0664	0.2385	0.0177	0.7119	0.0754	0.3025
Ornithine	-0.0490	0.4306	-0.0540	0.3376	-0.0588	0.2197	0.0031	0.9662
Phenylalanine	-0.0374	0.5478	-0.1527	0.0065	-0.0440	0.3584	0.1094	0.134
Proline	0.0187	0.7643	-0.1167	0.0379	-0.0011	0.9809	-0.0115	0.875
Serine	-0.0203	0.7437	0.0416	0.46	0.0059	0.9027	0.1121	0.1245
Threonine	-0.0732	0.2383	0.0167	0.7671	0.0663	0.1661	0.1180	0.1058
Tryptophan	-0.1049	0.0907	-0.0624	0.268	0.0045	0.9252	0.0825	0.2593
Tyrosine	-0.0649	0.2963	-0.0991	0.078	-0.0084	0.8606	0.0469	0.522
Valine	-0.0622	0.3172	-0.1628	0.0037	-0.0943	0.0485	-0.0159	0.8279
xLeucine	-0.0661	0.2874	-0.1789	0.0014	-0.0414	0.3872	-0.0297	0.6851
Hexose								
H1	-0.0455	0.4638	-0.1078	0.0552	-0.0249	0.6033	-0.0546	0.4553
Acylcarnitines								
C0	0.0605	0.3304	-0.0444	0.4308	-0.0296	0.5371	0.0099	0.8923
C2	0.0361	0.5621	-0.0130	0.8174	-0.0058	0.9045	0.0130	0.8588
C3	-0.0773	0.2134	-0.1280	0.0227	-0.0626	0.1912	0.0099	0.8927
C5-DC (C6-OH)	0.0113	0.8561	0.0235	0.6775	0.0175	0.7149	0.1229	0.092
C5-OH(C3-DCM)	0.0181	0.7715	-0.0126	0.8226	-0.0251	0.6011	0.0511	0.4854
C7-DC	0.0251	0.686	0.0467	0.4077	0.0868	0.0697	0.0759	0.2995
C8:1	0.0177	0.7765	0.0079	0.8892	-0.1090	0.0226	0.0396	0.5883
C9	0.0942	0.1292	0.0559	0.3214	0.0195	0.6834	0.0378	0.606
C10	0.0646	0.2988	0.1294	0.0212	0.0429	0.3705	0.0924	0.206
C16	0.0251	0.6868	-0.0521	0.3549	0.0471	0.3253	0.0662	0.3656
C18	0.0395	0.5252	0.0307	0.5861	0.0857	0.0732	0.1575	0.0305
C10:2	0.0623	0.3159	-0.0265	0.6381	0.0226	0.6368	0.0527	0.4717
C14:1	-0.0080	0.8975	0.0162	0.7737	0.0061	0.8989	0.0245	0.7384
C14:2	0.0386	0.5351	0.0753	0.1813	-0.0124	0.7955	0.0428	0.5589
C16:2	0.1088	0.0793	-0.0024	0.9666	0.0756	0.1144	0.0694	0.3427
C18:1	0.0071	0.909	0.0256	0.6497	0.0381	0.4259	0.0555	0.4485
C18:2	-0.0026	0.9673	-0.0216	0.7014	0.0261	0.5853	0.1223	0.0936
Diacyl-Phosphatidylcholines								
PC aa C28:1	0.0731	0.2394	-0.0295	0.6011	-0.0525	0.2733	0.0805	0.2711
PC aa C30:0	-0.0370	0.5522	-0.0662	0.2397	-0.0646	0.1773	0.0403	0.582
PC aa C32:0	-0.0175	0.7787	-0.0436	0.4393	-0.0782	0.102	0.0802	0.2724

Table continued on the next page

	Men				Women			
	Estradiol (n=261)		Testosterone (n=317)		Estradiol (n=438)		Testosterone (n=189)	
	r	p	r	p	r	p	r	p
PC aa C32:1	-0.0055	0.93	-0.0556	0.3239	-0.0544	0.2558	0.0633	0.3866
PC aa C32:2	-0.0073	0.9063	-0.1103	0.0499	-0.0742	0.1209	-0.0041	0.9556
PC aa C32:3	-0.0015	0.9804	-0.0096	0.8644	-0.0872	0.0682	-0.0054	0.9414
PC aa C34:1	-0.0107	0.8636	-0.0221	0.6951	-0.0368	0.442	0.0676	0.3556
PC aa C34:2	0.0011	0.9864	-0.0366	0.5165	-0.0786	0.1004	0.0379	0.6044
PC aa C34:3	-0.0013	0.9834	-0.0493	0.3813	-0.0669	0.1619	-0.0077	0.9158
PC aa C34:4	-0.0343	0.5813	-0.1671	0.0028	-0.0725	0.1296	-0.0024	0.9741
PC aa C36:0	0.0432	0.4871	0.0244	0.6658	0.0413	0.3889	0.1281	0.0791
PC aa C36:1	-0.0305	0.6235	-0.0001	0.9989	-0.0060	0.9	0.0768	0.2936
PC aa C36:2	-0.0391	0.5293	-0.0239	0.6712	-0.0151	0.7535	0.0966	0.1862
PC aa C36:3	-0.0101	0.8714	-0.0596	0.2904	-0.0908	0.0577	0.0413	0.5722
PC aa C36:4	-0.0468	0.4519	-0.1457	0.0094	-0.0646	0.177	0.0595	0.4161
PC aa C36:5	0.0169	0.786	-0.0908	0.1066	0.0135	0.7785	-0.0911	0.2127
PC aa C36:6	0.0322	0.6045	-0.1283	0.0223	-0.0172	0.7202	-0.0122	0.8673
PC aa C38:0	0.0239	0.7004	0.0659	0.2423	0.0178	0.7107	0.1208	0.0978
PC aa C38:1	0.0663	0.2861	0.0350	0.5343	0.1014	0.0339	-0.0075	0.9187
PC aa C38:3	-0.0360	0.5632	-0.1298	0.0208	-0.0989	0.0385	0.0464	0.5257
PC aa C38:4	-0.0961	0.1215	-0.1552	0.0056	-0.0293	0.5413	0.1004	0.1695
PC aa C38:5	-0.0207	0.7394	-0.1236	0.0278	0.0265	0.5797	0.0046	0.9505
PC aa C38:6	0.0629	0.3116	-0.1054	0.0608	0.0189	0.6936	0.0582	0.4263
PC aa C40:2	-0.0082	0.8953	0.0087	0.8771	-0.0304	0.5252	-0.0363	0.6196
PC aa C40:3	0.0343	0.5817	0.0162	0.7743	-0.0102	0.8316	-0.0105	0.8861
PC aa C40:4	-0.0460	0.4591	-0.0916	0.1036	-0.0261	0.5861	0.0541	0.4598
PC aa C40:5	-0.0126	0.8397	-0.1175	0.0366	0.0172	0.7198	0.0181	0.8045
PC aa C40:6	0.0237	0.7037	-0.1267	0.024	0.0076	0.874	0.0764	0.2964
PC aa C42:0	-0.0359	0.5637	0.0463	0.4113	0.0563	0.2396	0.0613	0.4025
PC aa C42:1	0.0028	0.9637	0.0116	0.8376	0.0380	0.4272	0.1111	0.1281
PC aa C42:2	-0.0180	0.7721	-0.0450	0.4251	-0.0115	0.8099	-0.0478	0.5139
PC aa C42:4	-0.0422	0.4971	0.0319	0.571	-0.0401	0.4025	0.0425	0.5619
PC aa C42:5	0.0584	0.3471	-0.0145	0.7971	0.0184	0.701	-0.0128	0.8616
PC aa C42:6	0.0098	0.8749	-0.0297	0.5987	0.0157	0.744	-0.0604	0.4091
Acyl-alkyl-Phosphatidylcholines								
PC ae C30:0	-0.0032	0.9587	0.0173	0.7597	0.0216	0.6522	0.0705	0.3351
PC ae C30:1	-0.1057	0.0884	-0.0160	0.7769	-0.0643	0.1794	-0.0041	0.9559
PC ae C30:2	-0.0704	0.2568	-0.0086	0.8783	-0.0486	0.31	0.1031	0.1581
PC ae C32:1	0.0149	0.8102	0.1360	0.0154	0.0272	0.5702	0.0421	0.5654
PC ae C32:2	0.0524	0.3994	0.0996	0.0767	0.0251	0.5998	0.1066	0.1445
PC ae C34:0	0.0359	0.5641	0.0369	0.5127	-0.0092	0.8472	0.0565	0.4396
PC ae C34:1	0.0183	0.7684	0.0887	0.115	-0.0098	0.8381	0.0565	0.4397
PC ae C34:2	-0.0126	0.8398	0.1153	0.0402	-0.0054	0.9099	0.0417	0.5693
PC ae C34:3	0.0337	0.5882	0.1451	0.0097	0.0145	0.763	0.0243	0.7396
PC ae C36:0	-0.0189	0.7615	0.0106	0.8514	0.0569	0.2347	0.0879	0.2289

Table continued on the next page

	Men				Women			
	Estradiol (n=261)		Testosterone (n=317)		Estradiol (n=438)		Testosterone (n=189)	
	r	p	r	p	r	p	r	p
PC ae C36:1	0.0830	0.1815	0.0486	0.389	-0.0112	0.8157	0.0536	0.4637
PC ae C36:2	0.0767	0.2166	0.0962	0.0874	0.0039	0.9358	0.0334	0.6487
PC ae C36:3	-0.0128	0.8374	0.1194	0.0337	-0.0206	0.6672	0.0595	0.4158
PC ae C36:4	-0.0480	0.4401	0.0027	0.9623	-0.0318	0.5069	0.1188	0.1034
PC ae C36:5	0.0374	0.5472	0.0481	0.3938	-0.0017	0.9712	0.1075	0.141
PC ae C38:0	0.0418	0.5013	-0.0492	0.3824	0.0246	0.607	0.0327	0.6549
PC ae C38:1	0.0165	0.7913	0.0519	0.3573	0.0085	0.8585	0.0542	0.4588
PC ae C38:2	0.0060	0.9232	0.0991	0.0782	0.0095	0.8422	0.0310	0.6722
PC ae C38:3	0.0431	0.4882	0.0267	0.6355	-0.0416	0.3852	0.0159	0.8283
PC ae C38:4	-0.0323	0.6039	0.0331	0.557	0.0183	0.7021	0.0776	0.2883
PC ae C38:5	-0.0509	0.4132	0.0485	0.3892	0.0062	0.8973	0.1000	0.1709
PC ae C38:6	0.0420	0.4991	0.0297	0.5989	0.0202	0.6733	0.0909	0.2135
PC ae C40:1	-0.0664	0.2849	-0.0439	0.4357	0.0085	0.8592	0.0930	0.203
PC ae C40:2	0.0699	0.2603	0.0479	0.3958	0.0128	0.7889	0.0203	0.7819
PC ae C40:3	0.0022	0.9719	0.0866	0.1237	0.0530	0.2682	0.0400	0.5848
PC ae C40:4	-0.0432	0.4876	0.0769	0.1719	0.0723	0.1307	0.0825	0.2594
PC ae C40:5	-0.0329	0.5972	0.0590	0.295	0.0605	0.2064	0.0269	0.7135
PC ae C40:6	0.0637	0.3052	0.0526	0.3507	0.0681	0.1548	0.0618	0.3986
PC ae C42:1	-0.1190	0.0548	-0.0447	0.4275	-0.0111	0.8169	0.1341	0.0658
PC ae C42:2	0.0396	0.5239	0.0029	0.9592	0.0823	0.0855	0.0702	0.3372
PC ae C42:3	-0.0063	0.9192	0.0442	0.4327	0.0632	0.187	0.0889	0.2238
PC ae C42:4	-0.0688	0.2685	0.1087	0.0533	0.0645	0.1777	0.0135	0.8542
PC ae C42:5	-0.0517	0.4057	0.0808	0.1512	0.0692	0.1485	0.0487	0.5062
PC ae C44:3	-0.0554	0.3723	-0.0194	0.7314	0.0330	0.4911	0.1556	0.0325
PC ae C44:4	-0.0714	0.2503	0.1251	0.0259	0.0441	0.3574	0.0107	0.8834
PC ae C44:5	-0.0837	0.1776	0.1122	0.046	0.0490	0.3058	0.0295	0.6873
PC ae C44:6	-0.0595	0.3383	0.0744	0.1865	0.0326	0.4965	0.0631	0.3882
Lyso-Phosphatidylcholines								
lysoPC a C14:0	-0.0757	0.2232	-0.1317	0.019	-0.0304	0.5264	-0.0208	0.7765
lysoPC a C16:0	-0.0493	0.428	-0.1282	0.0224	-0.0636	0.1841	0.0857	0.241
lysoPC a C16:1	-0.0149	0.8103	-0.0985	0.0799	-0.0284	0.5537	0.0422	0.5645
lysoPC a C17:0	0.0512	0.4101	-0.0191	0.7347	0.0121	0.8008	0.0125	0.865
lysoPC a C18:0	-0.0698	0.261	-0.0883	0.1168	-0.0161	0.7367	0.1158	0.1126
lysoPC a C18:1	-0.0747	0.2291	-0.0066	0.9071	0.0217	0.65	0.0622	0.3955
lysoPC a C18:2	-0.0654	0.2927	-0.0269	0.6334	0.0346	0.4697	0.0654	0.3713
lysoPC a C20:3	-0.1070	0.0846	-0.1583	0.0047	-0.0647	0.1763	0.0722	0.3236
lysoPC a C20:4	-0.1171	0.0588	-0.1425	0.0111	-0.0077	0.8732	0.1094	0.1341
lysoPC a C28:1	-0.1333	0.0313	-0.0315	0.5768	-0.0216	0.6516	0.1016	0.1643
Sphingomyelins								
SM C16:0	-0.0586	0.3456	0.0941	0.0945	0.0040	0.9333	0.0696	0.3415
SM C16:1	-0.0090	0.8855	0.0376	0.5051	-0.0422	0.3788	0.0055	0.9399
SM C18:0	-0.0445	0.474	0.0115	0.8391	-0.0384	0.4226	0.0823	0.2601

Table continued on the next page

	Men				Women			
	Estradiol (n=261)		Testosterone (n=317)		Estradiol (n=438)		Testosterone (n=189)	
	r	p	r	p	r	p	r	p
SM C18:1	-0.0314	0.6131	0.0128	0.8204	-0.0381	0.4261	0.0682	0.351
SM C20:2	0.0318	0.609	0.0417	0.4598	-0.0241	0.615	-0.0230	0.7531
SM C24:0	-0.1170	0.059	-0.0311	0.5817	-0.0859	0.0725	0.0058	0.937
SM C24:1	-0.0407	0.5127	0.0977	0.0823	-0.0414	0.3879	0.0115	0.8751
SM C26:0	-0.0531	0.3928	0.0627	0.2658	0.0404	0.3986	0.0195	0.7904
SM C26:1	-0.0707	0.2551	0.0237	0.6739	-0.0282	0.5556	-0.0052	0.9438
SM (OH) C14:1	0.0953	0.1247	0.0966	0.086	0.0003	0.9959	0.0711	0.331
SM (OH) C16:1	0.0354	0.5691	0.0889	0.1142	0.0068	0.8865	0.0826	0.2583
SM (OH) C22:1	-0.0560	0.3676	0.0317	0.5739	-0.0520	0.2775	0.0203	0.7819
SM (OH) C22:2	0.0137	0.8255	0.0866	0.1238	-0.0308	0.5199	0.0009	0.9905
SM (OH) C24:1	-0.0542	0.3831	0.0027	0.9622	-0.0185	0.6999	0.0345	0.6377

Abbreviations: PC, phosphatidylcholine; a, acyl; e, alkyl; SM, sphingomyelin