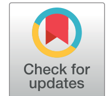

Effect of General Adiposity and Central Body Fat Distribution on the Circulating Metabolome: A Multicohort Nontargeted Metabolomics Observational and Mendelian Randomization Study

Shafqat Ahmad,[1,2] Ulf Hammar,[1] Beatrice Kennedy,[1] Samira Salihovic,[1,3] Andrea Ganna,[4,5] Lars Lind,[6] Johan Sundström,[7,8] Johan Ärnlöv,[9,10] Christian Berne,[1] Ulf Risérus,[11] Patrik K.E. Magnusson,[5] Susanna C. Larsson,[12,13] and Tove Fall[1]

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Obesity is associated with adverse health outcomes, but the metabolic effects have not yet been fully elucidated. We aimed to investigate the association between adiposity and circulating metabolites and to address causality with Mendelian randomization (MR). Metabolomics data were generated with nontargeted ultraperformance liquid chromatography coupled to time-of-flight mass spectrometry in plasma and serum from three population-based Swedish cohorts: ULSAM (*N* = 1,135), PIVUS (*N* = 970), and TwinGene (*N* = 2,059). We assessed associations of general adiposity measured as BMI and central body fat distribution measured as waist-to-hip ratio adjusted for BMI (WHRadjBMI) with 210 annotated metabolites. We used MR analysis to assess causal effects. Lastly, we attempted to replicate the MR findings in the KORA and TwinsUK cohorts (*N* = 7,373), the CHARGE Consortium (*N* = 8,631), the Framingham Heart Study (*N* = 2,076), and the DIRECT Consortium (*N* = 3,029). BMI was associated with 77 metabolites, while WHRadjBMI was associated with 11 and 3 metabolites in women and men, respectively. The MR analyses in the Swedish cohorts suggested a causal association (*P* value <0.05) of increased general adiposity and reduced levels of arachidonic acid, dodecanedioic acid, and lysophosphatidylcholine (P-16:0) as well as with increased creatine levels. The results of the replication effort provided support for a causal association of adiposity with reduced levels of arachidonic acid (*P* value = 0.03). Adiposity is associated with variation of large parts of the circulating metabolome; however, further investigation of causality is required in well-powered cohorts.

The increasing prevalence of obesity is a major health problem, as epidemiological as well as clinical evidence states that both general and central obesity constitute strong risk factors for several adverse health outcomes including cardiovascular disease (CVD) and type 2 diabetes

[1]Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[2]Preventive Medicine Division, Harvard Medical School, Brigham and Women's Hospital, Boston, MA
[3]School of Medical Sciences, Örebro University, Örebro, Sweden
[4]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA
[5]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
[6]Department of Medical Sciences, Cardiovascular Epidemiology, Uppsala University, Uppsala, Sweden
[7]Department of Medical Sciences, Clinical Epidemiology, Uppsala University, Uppsala, Sweden
[8]The George Institute for Global Health, Sydney, Australia
[9]Division of Family Medicine and Primary Care, Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet, Stockholm, Sweden
[10]School of Health and Social Studies, Dalarna University, Falun, Sweden
[11]Department of Public Health and Caring Sciences, Clinical Nutrition and Metabolism, Uppsala University, Uppsala, Sweden
[12]Unit of Cardiovascular and Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[13]Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

Corresponding author: Tove Fall, tove.fall@medsci.uu.se

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(1,2). The metabolic effects of increased adiposity are not yet fully understood. Metabolomics methods can detect and quantify small compounds such as sugars, amino acids, organic acids, nucleotides, and lipid molecules (up to ~1,500 Da) in biological samples and can be used to assess metabolic effects of different exposures. Contemporary metabolomics approaches include gas chromatography and ultraperformance liquid chromatography (UPLC) coupled with mass spectrometry–based methods (MS) or nuclear magnetic resonance (NMR) spectroscopy methods (3). The MS approach may entail greater sensitivity than NMR and thus has the potential for discovery of a larger number of metabolites (4). Previous metabolomics studies have shown that increased adiposity is associated with many different metabolites such as increased levels of circulating aromatic amino acids, branched-chain amino acids, and certain fatty acids (5–7).

Mendelian randomization (MR) is a framework to study the causal effects of modifiable exposures on different phenotypes, where genetic variants are used as instrumental variables for the exposures of interest. As the random assortment of genetic variants occurs at the time of conception, these variants are not affected by confounding or reverse causation (8). Previous MR studies examining the causal relationship between general adiposity, which was measured as BMI, and metabolomics as measured by NMR (9,10), have indicated that increased adiposity has an impact on the levels of multiple circulating metabolites, including lipoproteins, branched-chain and aromatic amino acids, and inflammation-related glycoprotein acetyls. A recent study in TwinsUK (mainly females) (7) found that up to one-third of metabolites measured by MS methods were associated with obesity. However, in the genetic analysis, the BMI-associated genetic risk score based on 97 BMI-predisposing variants was not found to be associated with any specific metabolite.

Another approach to investigate causal effects of adiposity on the metabolome is through repeated measurements of the metabolome in weight loss trials. In two weight loss intervention studies—where one studied calorie restriction only and the other focused on calorie restriction only, calorie restriction combined with physical activity, or calorie restriction combined with a centrally acting serotonin-norepinephrine reuptake inhibitor approved for weight loss—it was found that weight loss was associated with increased plasma levels of medium- and long-chain acylcarnitines (11,12). Results of another study, of 57 women allocated to an intensive lifestyle weight loss program or control group, showed that 3 months of lifestyle intervention led to higher levels of 3-hydroxybutyrate, formate, methylguanidine, *myo*-inositol, and phosphocreatine as well as lower levels of proline and trimethylamine (13).

However, more studies of the effect of general adiposity and body fat distribution on the circulating metabolome are necessary for better understanding of the metabolic consequences of obesity. The aim of the current study was therefore twofold: *1*) to identify circulating metabolites measured with MS associated with adiposity and central body fat distribution and *2*) to investigate whether these associations were due to a causal effect of adiposity.

RESEARCH DESIGN AND METHODS

Cohorts

Three population-based Swedish cohorts were used. The Uppsala Longitudinal Study of Adult Men (ULSAM) cohort was comprised of male residents born between 1920 to 1924 in Uppsala, Sweden, at age 50 years (N = 2,322) and includes several in-person assessments over nearly 50 years (14). In the current study we use information and metabolomics profiling from the 1,135 individuals participating in the investigation at age 70 years. In the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) (N = 970), a random sample was enrolled of both women and men, at age 70 years, from the residents of Uppsala in 2001. Participants were invited for repeated tests at ages 75 and 80 years also, and metabolomics profiling was performed on the 970 samples from the age 70 years assessment. The TwinGene cohort includes a total of 12,591 twins born in Sweden before 1958 (15). In the current study, samples from 2004 to 2008 (N = 2,059) underwent metabolomics profiling, with complete information on covariates, mean ± SD age 68.6 ± 8.3 years (16). In the subsampling strategy only one twin per pair was prioritized, and few complete twin pairs were therefore included (16). Metabolite measurements underwent a log2-transformation followed by SD transformation.

Cooperative Health Research in the Region of Augsburg (Kooperative Gesundheits-forschung in der Region Augsburg [KORA]) is a population-based cohort from southern Germany that includes prospectively measured health assessment and blood samples collections between 2006 and 2008 (17). The current study (KORA F4) is based on 1,768 participants, mean ± SD age 60.8 ± 8.8 years, for whom the metabolomics assessment were performed. TwinsUK comprises a mainly (93%) female cohort of twins recruited from the U.K. The current study is based on 6,056 participants with mean age 53.4 ± 14.0 years who were analyzed for the metabolite profiling (17). Summary genome-wide association study (GWAS) data from meta-analysis of KORA and TwinsUK (17) were obtained from public repositories and were expressed as the per-allele log10-unit change in metabolite levels.

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium GWAS for plasma phospholipid fatty acid fractions was based on samples from 8,631 individuals (age range 45.8–72.0 years, 55% women) from the five epidemiological cohorts (18). In the CHARGE Consortium, arachidonic acid was expressed as percentage of total fatty acids.

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The Framingham Heart Study (FHS) is a population-based cohort of European ancestry participants (age range 45.0–65.0 years, 51% women) from the U.S. Samples from 2,076 FHS study participants underwent plasma metabolite and GWAS profiling. In the FHS cohort, GWAS analysis were conducted with use of normalized residuals of metabolites levels and linear mixed-effects models accounting for age and sex (19).

The DIabetes REsearCh on patient straTification (DIRECT) Consortium (N = 3,029) includes the participants with prediabetes (age range 56.0–68.0 years, 24% women) and type 2 diabetes (age range 56.0–68.1 years, 42% women) from the population-based cohorts across Europe (20). In the DIRECT Consortium, metabolite levels were expressed as residuals from a linear mixed model accounting for technical variables.

All participants provided written informed consent prior to inclusion in the respective cohort study, and the research was approved by the ethics committees of Uppsala University (ULSAM, PIVUS) and Karolinska Institutet (TwinGene) or the respective institutional review boards for the replication cohorts. The study was conducted according to the principles of the Declaration of Helsinki.

Anthropometric Measurements

Across all three Swedish cohorts, height was objectively measured to the nearest centimeter and body weight to the nearest 0.1 kg. BMI was calculated as weight in kilograms divided by the square of height in meters and used as a proxy for general adiposity. The waist was measured as a midway between the lowest rib and the iliac crest, while the hip circumference (HC) were measured over the widest part. Waist-to-hip ratio (WHR) was calculated as the ratio of waist circumference (WC) in centimeters to HC in centimeters and used as a proxy for central body fat distribution.

Measurement of Metabolites

In all Swedish cohorts, samples for metabolite assessment were taken on the same day as the anthropometric measurements. Blood was drawn from the study participants with overnight fasting in all cohorts.

Swedish Cohorts

The samples were treated with methanol for protein precipitation. Nontargeted metabolite profiling was carried out with UPLC (ACQUITY UPLC equipped with an ACQUITY UPLC BEH C8 analytical column [1.7 μm, 2.1 mm × 100 mm] coupled to a time-of-flight mass spectrometer [Waters Corporation, Milford, MA]) with an electrospray source operated through positive-ion mode. For quality control (QC), prior to each batch of two 96-well plates of samples, instrument maintenance (cone cleaning, mass calibration, and detector gain calibration) was performed, and an external QC standard mix was injected containing 2 μg/mL each of caffeine, terfenadine, sulfadimethoxime, and reserpine. The QC standards were evaluated for retention time (±0.05 min), signal intensity (<25% relative standard deviation), and mass accuracy (<3 ppm). All samples were randomized prior to instrumental analysis. Since internal standards were not available at the time of analysis, randomized duplicate injections were performed to mitigate potential within-sample variation originating from the instrumental analysis. Average peak areas of the duplicate injections were then used for the relative quantitation.

Details of the metabolomics measurement procedures across these cohorts can be found in Supplementary Material and have also previously been published (16). After peak detection and data processing, metabolomics features were matched between cohorts based on retention time, accurate mass, and MS/MS spectra. After data processing and adjustment for technical factors, intensities were scaled to SD units prior to statistical analyses. The entire processing pipeline for metabolomics measurement has previously been described (16). Annotation of metabolites was performed with use of the in-house spectral library of authenticated standards and several publicly available spectral databases in accordance with the Metabolomics Standards Initiative (MSI). The level of confidence to which a positive metabolite annotation has been performed was categorized as follows: level 1, match with accurate mass (±5 ppm), fragmentation pattern, and retention time with the in-house spectral library containing >930 authentic standards collected under the same experimental conditions; level 2, match based on accurate mass and fragmentation pattern with use of available mass spectra in public databases without retention time information; and level 3, match based on a combination of mass spectra and fragmentation pattern knowledge, accurate mass, and retention time window for assignment of the metabolite to a chemical class/formula. In total, 106 of 220 metabolites were assigned at MSI level 1, 98 of 220 were assigned MSI level 2, and 4 of 220 metabolites were assigned MSI level 3.

Replication Cohorts

Metabolites were assessed in serum or plasma with liquid chromatography–MS/MS (LC-MS/MS) applying the Metabolon platform in KORA, TwinsUK, and DIRECT (17,20). In the CHARGE Consortium, arachidonic acid was measured in plasma phospholipids through thin-layer gas chromatography (except in the Invecchiare in Chianti [InCHIANTI] study where arachidonic acid was directly measured by gas chromatography) (18) and expressed as percentage of total plasma fatty acids. In the FHS cohort, plasma creatine metabolite was quantified through the LC-MS/MS method using a triple quadrupole mass spectrometer (Applied Biosystems/SCIEX) (19).

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Statistical Analyses

Observational Analyses of the Association of General Adiposity With Metabolites

The analysis plan and cohorts used are described in Fig. 1. We used a series of linear regression models to assess the association between general adiposity measured as BMI and the annotated metabolites from the ULSAM and PIVUS cohorts. In PIVUS, models were adjusted for age and sex, while for the ULSAM cohort the models were adjusted for age only; ULSAM includes only men.

The β-coefficients from the two cohorts were meta-analyzed using the DerSimonian-Laird random-effects model (21); details can be found in Supplementary Text 2. The random-effects model was chosen because initial analysis indicated considerate heterogeneity in effect estimates between cohorts.

A 5% false discovery rate (FDR) with the Benjamini-Hochberg procedure (22) was used to account for multiple testing. Metabolites that passed the FDR threshold were assessed in TwinGene. A similar model was used but with cluster-robust SEs to account for dependency within twins. Of 2,059 individuals, 75 complete twin pairs were included. Metabolites were considered replicated in TwinGene if the estimates were directionally similar and showed a P value of <0.05.

Observational Analyses of the Association of Central Body Fat Distribution With Metabolites

Similarly, we used a series of linear regression models to assess the association between body fat distribution measured as WHR and the annotated metabolites in the ULSAM and PIVUS cohorts. All models were also adjusted for age and BMI and run separately in men and women, as the distribution of WHR is sex specific. This model is hereafter referred to as "WHRadjBMI." For men, estimates from ULSAM and PIVUS samples were pooled and meta-analyzed with the DerSimonian-Laird random-effects model. For women, only PIVUS samples for WHR-metabolites association analyses were used in the first step, as ULSAM is men only. The Benjamini-Hochberg procedure (22) at a 5% FDR was again used to account for multiple testing. Metabolites that passed the FDR threshold were assessed in TwinGene. A similar model was used, but cluster-robust SEs were used to account for dependency within twins. Of 2,059 individuals, 75 twin pairs were included. Metabolites were considered replicated in TwinGene if estimates were in the same direction and showed a P value of <0.05. As a sensitivity analysis, we used WC, HC, and unadjusted WHR as alternative measures for body fat distribution. We assessed the association of WC and HC with metabolites identified in the main analysis adjusting for weight, height, and age using

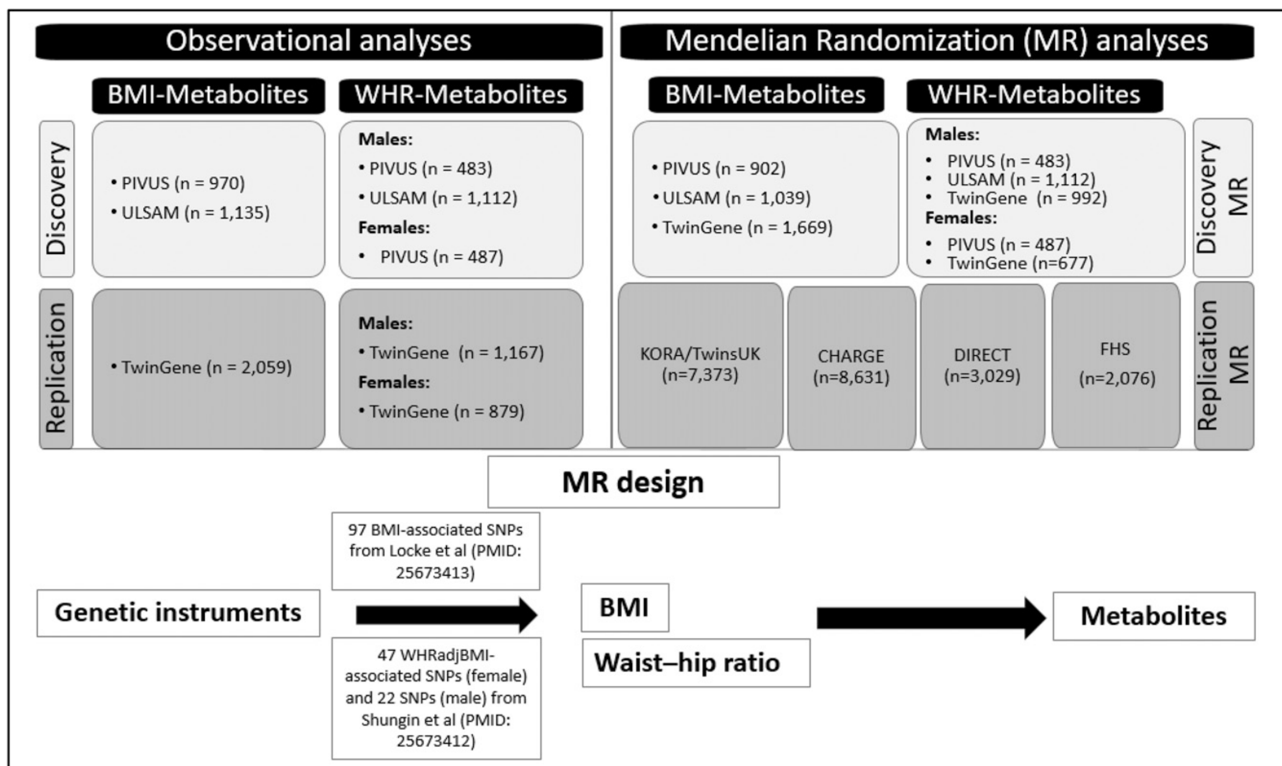

Figure 1—Schematic flow and cohorts used in the current analysis.

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the same sex-stratified strategy as for WHRadjBMI, while WHR was only adjusted for age.

MR

Metabolites associated with BMI in the observational analyses were taken forward to MR analyses. We performed a two-sample MR analysis where the association between the genetic instrument and adiposity was based on previous large GWAS studies (23,24) and the association between the genetic instrument and each metabolite was assessed in ULSAM, PIVUS, and TwinGene.

We created three genetic instruments, one for BMI (sex combined) and two sex-specific instruments for WHRadjBMI (male and female) based on two large GWAS studies (23,24). BMI and WHRadjBMI were expressed as z scores in these GWAS, derived from inverse normal transformation of residuals from a regression with adjustment for age, age squared, and study-specific covariates if necessary. The WHR phenotype was additionally adjusted for BMI. For the BMI instrument, we included independent single nucleotide polymorphisms (SNPs) showing association (P value $<5 \times 10^{-8}$) with BMI in sex-combined analysis (23). For each of the two sex-specific WHRadjBMI instruments, we included SNPs that showed association with WHRadjBMI if they had *1)* P value $<5 \times 10^{-8}$ in sex-combined and P value $<2.5 \times 10^{-4}$ in sex-specific analysis or *2)* P value $<5 \times 10^{-8}$ in sex-specific analysis. The BMI instrument thus included 97 SNPs, the female WHRadjBMI instrument included 47 SNPs, and the male WHRadjBMI instrument included 22 SNPs. We extracted estimates from the European ancestry sample for SNP-BMI from Table 1, Extended Table 2, and S4 Euro Sex Combined in the study by Locke et al. (23) and for SNP-WHRadjBMI from Table 1 and ST4 in the study by Shungin et al. (24). The included SNPs for BMI (sex-combined) and WHRadjBMI (sex-stratified) and their allele frequencies in TwinGene, PIVUS, and ULSAM are reported in Supplementary Tables 1, 2, and 3, respectively. Variants were extracted using PLINK 2.0 (https://pngu.mgh.harvard.edu/~purcell/plink/) from ULSAM, PIVUS, and TwinGene. Alleles were aligned to the reported obesity-increasing allele. We used both directly genotyped SNPs and imputed SNPs with HapMap imputation (ULSAM and PIVUS cohorts) or 1000 Genomes imputation panel (TwinGene) variants. All SNPs had minor allele frequency >1%, Hardy-Weinberg P value >0.01, and MACH2 imputation metric >0.95.

We investigated the association of each SNP with each metabolite using linear regression models in each Swedish cohort separately, with subsequent random-effects meta-analysis across all three Swedish cohorts. The genetic effects were assumed to be additive. All analyses were adjusted for age, sex, and the first four genetic principal components from each of three cohorts. Cluster-robust SEs (twin pair as cluster) were used in the TwinGene cohort. For WHRadjBMI, analyses were performed separately among women and men.

The multiplicative random-effects inverse variance weighted (IVW) (25) method was used to estimate causal effects as the main MR analysis. MR-Egger and weighted median regression (WMM) methods were used as sensitivity analyses (25). The MR-Egger method can be used to detect and adjust for directional pleiotropy, while the robust WMM method provides consistent estimates as long as at least 50% of the weights are based on valid (nonpleiotropic) instrumental variables (25,26). Details about the MR methods are described in Supplementary Text 3. MR analyses were performed with the Mendelian-Randomization package in R studio (R, version 3.6.0 [https://www.r-project.org/]). In MR analyses, we considered P values <0.05 to be statistically significant.

Pathway Enrichment Analysis

As an extended analysis, we performed enrichment analysis using the fast gene set enrichment analysis (FGSEA) tool to identify groups of metabolites enriched for genetic associations of obesity variants with single metabolites. We followed the same MR pipeline as previously, except that no observational analysis was used for filtering out nonsignificant metabolites. The P values from the MR analysis from the Swedish cohorts were then carried forward to FGSEA as implemented in the R package fgsea. At least 10 metabolites had to be present in a metabolite class for the FGSEA analysis to be performed.

Replication

We attempted replication of MR results for metabolites with P value <0.05 in the Swedish cohorts. Each replication cohort only had a few of the metabolites of interest available. The SNP-metabolite associations from replication cohorts were aligned to the BMI-increasing allele. Causal MR estimates were obtained using IVW, while the MR sensitivity analyses were performed through MR-Egger and WMM methods. We applied the Stouffer P value–based meta-analysis (27) to pool z scores from each replication cohort derived from one-sided P values, accounting for directionality of the individual cohort estimate.

Statistical analyses were performed with Stata 15.0 (Stata, College Station, TX) and R studio (R, version 3.6.0) unless otherwise noted.

Data and Resource Availability

The data that support the findings of this study from ULSAM, PIVUS, and TwinGene are available, but restrictions apply to the availability of these data, which were used under license for the current study and therefore are not publicly available. Data are, however, available from the authors on reasonable request and with permission of ULSAM, PIVUS, and TwinGene steering committees and with permission of the Swedish Ethical Review Authority. Data from KORA and TwinsUK are available here from https://metabolomics.helmholtz-muenchen.de/gwas/. Data

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from the CHARGE Consortium are publically available for download from https://www.msi.umn.edu/~wguan/CHARGE_N6GWAS/. Summary data for the association of SNPs with creatine were retrieved from the FHS (19). GWAS-metabolites data from the DIRECT Consortium are publically available (20).

RESULTS

Baseline characteristics of the study participants are reported in Table 1.

Observational Analyses of the Association of General Adiposity With Metabolites

In the meta-analysis of PIVUS and ULSAM, we found that BMI was associated with 109 of 210 tested metabolites (Supplementary Table 4), whereof 77 metabolites were replicated in TwinGene (Supplementary Table 5). Of these 77 metabolites, BMI was inversely associated with 13 of 15 lysophosphatidylcholine (LysoPC) and positively associated with 8 of 8 glycerolipids, 6 of 7 unsaturated fatty acids, 4 of 6 amino acids, peptides, and derivatives, 3 of 3 carnitines and acyl carnitines, and 5 of 5 bile acids.

Observational Analyses of the Association of Central Body Fat Distribution With Metabolites

In the meta-analysis of men from PIVUS (N = 483) and ULSAM (N = 1,112), WHRadjBMI was associated with 32 metabolites (Supplementary Table 6), whereof only 3 metabolites were replicated in TwinGene (N = 1,167) (Supplementary Table 7). In women from PIVUS (N = 487), WHRadjBMI was associated with 47 metabolites. Eleven of those metabolites were replicated in the female sample of TwinGene (N = 879). Of these, positive associations were found for 7 of 7 glycerolipids (Supplementary Table 7).

In the sensitivity analyses, most associations were comparable for the alternate exposures WC and unadjusted WHR with the exception of sphingomyelin(32:2). The association of sphingomyelin(32:2) with WHRadjBMI seems driven by association with HC adjusted for weight and height rather than WC (Supplementary Table 8).

MR: General Adiposity

In the meta-analysis of estimates in the ULSAM, PIVUS, and TwinGene cohorts, we found results consistent with a causal effect of general adiposity reducing levels of arachidonic acid, dodecanedioic acid, and LysoPC (P-16:0) (Table 2). We also observed evidence consistent with a causal effect of general adiposity on increased creatine levels (Table 2). We observed that dodecanedioic acid and LysoPC (P-16:0) had a strong negative correlation with BMI, while arachidonic acid and creatine were positively correlated (Supplementary Table 9).

MR: Body Fat Distribution

We found evidence of a causal effect of central body fat distribution on decreased levels of sphingomyelin(32:2) in women (Table 2). No findings were present in men.

Pathway Enrichment Analysis

We were unable to identify any enrichment for metabolite class-wise associations for BMI or WHRadjBMI in the MR (Supplementary Table 10).

Replication

Next, we attempted to replicate MR results in four independent studies with data for three of the metabolites with $P < 0.05$ in the Swedish cohorts. Genetic association results were available for arachidonic acid and creatine in three cohorts and for dodecanedioic acid in one cohort. We observed directionally consistent results for all tested metabolites in all

Table 1—Clinical characteristics of the ULSAM, PIVUS, and TwinGene Swedish cohorts

	ULSAM	PIVUS	TwinGene
N total	1,135	970	2,059
% men	100	50	58
Age, years	70.9 ± 0.6	70.2 ± 0.2	68.6 ± 8.3
BMI, kg/m^2	26.3 ± 3.4	27.1 ± 4.3	26.3 ± 4.0
WHR	0.95 ± 0.05	0.90 ± 0.7	0.91 ± 0.1
Waist (cm)	94.9 ± 9.6	90.1 ± 15.7	93.6 ± 11.9
Diastolic blood pressure (mmHg)	79.6 ± 8.6	78.6 ± 10.2	82.0 ± 10.6
Systolic blood pressure (mmHg)	140.0 ± 16.3	149.7 ± 22.6	142.7 ± 20.2
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.5 ± 0.4	1.4 ± 0.4
LDL cholesterol (mmol/L)	3.9 ± 0.9	3.4 ± 0.9	3.7 ± 1.0
Triglycerides (mmol/L)	1.5 ± 0.8	1.3 ± 0.6	1.4 ± 0.8
Total cholesterol (mmol/L)	5.8 ± 1.0	5.4 ± 1.0	5.7 ± 1.2

Data are means ± SD unless otherwise indicated.

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Table 2—Observational and causal associations between BMI and metabolites across the combined sample (N = 3,610) of ULSAM, PIVUS, and TwinGene and causal WHRadjBMI and metabolomics signatures among women in the combined sample (N = 1,158) of PIVUS and TwinGene

	Observational analysis				MR									
	ULSAM and PIVUS		TwinGene		Swedish cohorts		KORA/TwinsUK		CHARGE Consortium		DIRECT Consortium		FHS	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Metabolite														
Dodecanedioic acid	−0.04 (−0.05, −0.02)	4.1E−10	−0.02 (−0.04, −0.01)	3.8E−06	−0.35 (−0.57, −0.12)	2.0E−03	−0.03 (−0.06, 0.004)	0.25	NA		NA		NA	
LysoPC (P-16:0)	−0.05 (−0.07, −0.04)	3.2E−09	−0.04 (−0.05, −0.03)	3.4E−11	−0.29 (−0.52, −0.05)	0.02	NA		NA		NA		NA	
Arachidonic acid	0.02 (0.01, 0.03)	4.4E−03	0.02 (0.01, 0.04)	1.3E−04	−0.26 (−0.48, −0.04)	0.02	−0.01 (−0.03, 0.01)	0.48	−0.25 (−0.51, 0.003)	0.05	−0.03 (−0.16, 0.20)	0.60	NA	
Creatine	0.02 (0.01, 0.03)	6.0E−03	0.02 (0.01, 0.03)	5.9E−03	0.25 (0.01, 0.50)	0.04	0.02 (−0.01, 0.05)	0.13	NA		0.07 (−0.06, 0.09)	0.30	−0.21 (−0.49, 0.08)	0.16
WHRadjBMI-associated metabolite (females): sphingomyelin(32:2)	−2.46 (−3.78, −1.14)	3.0E−04	−0.71 (−1.30, −0.12)	0.02	−0.51 (−0.90, −0.13)	8.0E−03	NA		NA		NA		NA	

The table also includes validation of BMI-associated metabolites in the KORA/TwinsUK cohorts (N = 7,373), the CHARGE Consortium (N = 8,631), the DIRECT Consortium (N = 3,029), and the FHS cohort (N = 2,076). MR estimates were retrieved using the IVW method. Units of metabolites are not similar in discovery and replication cohorts. For the SNP-metabolites association analysis, in the Swedish cohorts log2 and then SD transformation was applied. In the KORA/TwinsUK cohorts for GWAS-metabolites analysis, log10-unit change in metabolite levels was used. In the CHARGE Consortium, GWAS results for SNP association with arachidonic acid are expressed as percentage of total fatty acids. In the DIRECT Consortium, GWAS-metabolites association analyses were residualized after removal of technical covariates with a linear mixed model. In the FHS cohort, GWAS-creatine analyses were performed using normalized residuals of creatine levels, with adjustment for age and sex. NA, not available.

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cohorts compared with the Swedish cohorts except for creatine in FHS (Table 2). Meta-analysis provided support of replication for causal effect of adiposity in lowering arachidonic acid ($P = 0.03$) but not for creatine ($P = 0.25$).

Sensitivity Analyses

The sensitivity analysis using MR-Egger and WMM yielded wide CIs but with point estimates in general agreeing with the IVW method. For arachidonic acid, we observed directionally similar β-coefficients for WMM and MR-Egger methods in the Swedish cohorts (Supplementary Table 11), KORA/TwinsUK, and the CHARGE Consortium but not in the DIRECT Consortium compared with IVW estimates (Supplementary Table 12). For creatine, we observed directionally consistent β-coefficients for WMM and MR-Egger methods in the Swedish cohorts (Supplementary Table 11), KORA/TwinsUK, DIRECT (not for MR-Egger), and the FHS (not for WMM) compared with IVW estimates (Supplementary Table 12). Supplementary Fig. 1*A*–*D* show scatter plots of the ratio estimates for genetic associations between SNP-BMI and SNP-metabolites among the Swedish and the replication cohorts when available, which allow visualization of the causal effect estimates. Supplementary Fig. 2*A* represents scatter plots of the ratio estimates for genetic associations between SNP-WHRadjBMI and SNP-metabolites [sphingomyelin(32:2)] among the Swedish cohorts. The mean Spearman correlation between duplicate injections across samples was 0.43 in PIVUS, 0.38 in TwinGene, and 0.46 in ULSAM. The mean feature correlations for the top BMI-associated metabolic features are provided in Supplementary Table 13 and ranged from 0.48 to 0.87. Supplementary Figs. 3–7 show raw data agreement in terms of retention time, mass accuracy, mass spectra, annotation confidence, and detection frequencies (missingness) across the three Swedish cohorts of PIVUS, ULSAM, and TwinGene.

DISCUSSION

In this study of middle-aged and elderly men and women, we found associations of BMI with large parts of the measured metabolome. We identified 77 BMI-metabolite associations, 11 WHRadjBMI-metabolite associations in women, and 3 WHRadjBMI-metabolite associations in men. We noted that the direction of association was similar within chemical classes of metabolites. Of the 77 BMI-associated metabolites, BMI was inversely associated with 13 of 15 LysoPC and positively associated with 8 of 8 glycerolipids, 6 of 7 unsaturated fatty acids, 4 of 6 amino acids, peptides, and derivatives, 3 of 3 carnitines and acyl carnitines, and 5 of 5 bile acids. Our findings further provided some evidence of causal association of general adiposity with lower levels of arachidonic acid.

Comparison With Literature

In a previous (28) study with comparison of metabolites, measured with MS, with different measures of adiposity, investigators found that metabolites showed similar relationships with BMI and WC adjusted for HC. Their main findings included positive associations with four amino acids and two sphingomyelins, negative correlations with LysoPC, and mixed directions for phosphatidylcholines. In our study, we adjusted all WHR analyses for BMI, which may explain that few metabolites overlapped between the phenotypes.

Unsaturated Fatty Acids

In observational analyses, we found positive associations between BMI and six of seven unsaturated fatty acids including arachidonic acid. Arachidonic acid, a derivative of linoleic acid, is an n-6 polyunsaturated fatty acid generally present in the human cell membrane, and the majority of arachidonic acid–related metabolites are proinflammatory (29).

However, our MR analyses suggested an inverse association of adiposity with arachidonic acid. In the Swedish cohorts, and in KORA/TwinsUK as well as the DIRECT Consortium, the levels of arachidonic acid represent free circulating arachidonic acid, while in the CHARGE Consortium the percentages of arachidonic acid were measured in phospholipids. The discrepancy in the direction of observational and MR analyses effect estimates might be explained by bidirectional effects or by confounding of the observational effect estimate. Previously published literature about the relationship between obesity and circulating arachidonic acid is inconclusive. Some studies report higher plasma phospholipid arachidonic acid in obese children (30) and adults (31), and others have reported lower erythrocyte phospholipid arachidonic acid in obese (32). The potential biological mechanisms for how genetic predisposition to higher BMI would lower circulating arachidonic acid indicated in our MR analyses are unclear. However, we speculate that it might involve lowered amounts of the enzyme delta-5-desaturase (D5D), which is encoded by the *FADS1* gene and converts the precursor dihomo-γ-linolenic acid into arachidonic acid. Surrogate measures of D5D activity were indeed found to be negatively correlated with BMI when assessed in circulating phospholipids (33,34). Other regulators of arachidonic acid levels are the amount of linoleic acid and the enzymes delta-6-desaturase, encoded by the *FADS2* gene, and elongases.

We found some evidence of a causal effect of general adiposity on reduced dodecanedioic acid levels in the Swedish cohorts data, but the results were not replicated in external data. Reduced levels of dodecanedioic acid have been observed in obese children compared with nonobese control subjects (5). Dodecanedioic acid is a water-soluble even-numbered dicarboxylic acid and structurally similar to medium-chain free fatty acids.

Amino Acids, Peptides, and Derivatives

In observational analyses, BMI was positively associated with four of the amino acids, peptides, and derivatives and

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negatively associated with two of the metabolites from this class. In observational and MR analyses, BMI was positively associated with creatine in the Swedish cohort, but these MR results were not replicated in external data. Creatine is an important metabolite for cellular energetics within skeletal muscle and plays a critical role in muscle catabolism during different physiological processes through increasing the ability of muscles to generate more ATP from ADP to match energy demands (36). Increasing body fat percentage, BMI, and WC have previously been associated with higher creatine levels (37), likely due to larger muscle mass. Similarly, in a lifestyle intervention study, weight loss was associated with decreased creatine (13). Hence, our observational and MR results in the Swedish cohorts data align well with previous reports, but MR results need to be confirmed.

Sphingomyelins

Sphingomyelin is one of the major lipids in the mammalian plasma membrane, and changes in membrane lipid composition change not only structure but also membrane function and receptor reorganization (38). In a previous study of the sphingomyelin profile of Korean middle-aged men with early stages of diabetes, most species of sphingomyelins were increased in subjects with abdominal obesity compared with those who were lean (39). In the current study, we observed that BMI was associated with reduced levels of two sphingomyelins. Similarly, in the current study we found preliminary evidence for increased central body fat distribution lowering sphingomyelin(32:2) in women, but no external replication data were available. However, sensitivity analysis using alternative measures of body fat distribution indicated that the identified association with WHRadjBMI was driven by increased HC rather than reduced WC. We are not aware of any similar studies in women, and our findings need to be validated before further conclusions are drawn.

LysoPC

LysoPC are the major components of oxidized LDL cholesterol. In the current study, we found inverse associations between BMI and 13 LysoPC and positive association with 2. In MR analyses in the Swedish cohorts data, we found evidence that increased adiposity was causally associated with reduced levels of LysoPC (P-16:0). However, no replication data were identified, and these results need confirmation. Most observational studies have reported inverse associations between adiposity and LysoPC. Reduced LysoPC levels have been observed after weight loss (40), while in other studies investigators observed nonsignificant differences in LysoPC species following weight reduction (11). LysoPC (P-16:0) levels have positively been associated with neonatal birth weight (41).

Strengths and Limitations

The strengths of the study include the combination of cross-sectional analysis with MR analyses and objectively assessed anthropometric measures. However, a few potential limitations deserve mentioning. First, the study size only yielded moderate statistical power to detect causal effects of obesity on metabolites, and also the nontargeted metabolomics approach only captured a limited set of metabolites. Replication data availability was limited. Second, all analyses were conducted in Europeans who were of older age, and therefore our results may have limited generalizability to other ethnicities and age-groups. The limited number of annotated metabolites are due to the single liquid chromatography setting and by somewhat limited availability of in-house standards. Another limitation is related to the measurements of central body fat distribution. We explored alternative measures of body fat distribution for our top findings from the main analysis. We observed comparable results for most metabolites with the alternative waist-related measures but few associations with HC supporting waist-related adiposity as the main link to these metabolites. Sphingomyelin(32:2) was the exception to this pattern where the inverse associations with WHRadjBMI were driven by increased HC. Furthermore, although the causal associations between adiposity and arachidonic acid were mostly consistent across different MR methods, our findings require validation using physiological models, particularly to rule out any potential biases related to unmeasured horizontal pleiotropy and canalization. Another potential limitation is that the nontargeted metabolomics platform was biased toward detection of metabolites that can be separated with reverse-phase liquid chromatography and that are readily detected in positive electrospray ionization mode, i.e., various phospholipids, fatty acids, acylcarnitines, and nonpolar amino acids and their derivatives as well as several other semipolar metabolites such as, for example, imidazopyrimidines (caffeine and theobromine) and indoles (indolelactic acid, 3-indolepropionic acid). Another potential limitation is that the external validation data set DIRECT Consortium includes data of participants with prediabetes and patients having newly diagnosed type 2 diabetes, while the participants of all other cohorts were predominantly disease free. Since metabolites were measured on a relative scale and with different normalization procedures in Swedish cohorts (ULSAM, PIVUS, and TwinGene), KORA/TwinsUK cohorts, CHARGE Consortium, FHS cohort, and DIRECT Consortium, effect size comparison between the Swedish cohorts, KORA/TwinsUK, the CHARGE Consortium, and the DIRECT Consortium is not possible; hence, *P* value–based meta-analysis was performed. The effect estimates were in general larger for WHRadjBMI compared with BMI. This is due to the difference in the unit of measurement for BMI and WHR. For SNP-BMI and WHRadjBMI association analysis, z score transformation was used. Some metabolite measurements may be affected by difference in matrix (plasma or serum), which may have limited the power of the study. In our previous studies, we have, however, been able to

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replicate a large proportion of associations with disease phenotypes across the three Swedish cohorts (42,43). Lastly, although we aimed to assess the causality between adiposity and annotated metabolites, we did not assess reverse causality, mainly because no specific genetic variants are available for the majority of studied metabolites.

Conclusions

In summary, we confirmed previous findings that adiposity is associated with large parts of the circulating metabolome. In the MR analysis, our findings suggested a causal association of increased general adiposity and reduced levels of arachidonic acid. Our findings regarding arachidonic acid need to be verified in a larger sample and investigated in relation to cardiometabolic disease for an understanding of whether they represent pathways of obesity causing diabetes and cardiovascular disease. Circulating arachidonic acid was not associated with future risk of CVD in a large observational study (44). However, plasma phospholipid arachidonic acid has shown some evidence for increasing risk of ischemic heart disease, ischemic stroke, and peripheral artery disease in an MR analysis, but our results do not support that obesity aggravates this pathway, as our results indicate that obesity lowers circulating arachidonic acid (45). Although we observed robust observational associations of BMI with a large number of metabolites, the explained variance of these circulating metabolites was in general low. In summary, larger studies including genetic data, metabolomics, anthropometric measurements, and follow-up for incident diabetes and cardiovascular events are needed to tease out whether metabolites mediate some of the increased risk of cardiometabolic disease in obesity.

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Duality of Interest. J.Ä. has served on advisory boards for AstraZeneca and Boehringer Ingelheim and has received lecturing fees from AstraZeneca and Novartis, all unrelated to the present project. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. S.A. contributed to the study design, conducted statistical analysis, and contributed to writing the first draft of the manuscript. U.H. contributed to the study design and conducted statistical analysis. S.S. assisted in the data analysis and metabolomics annotation and contributed to the writing of manuscript. L.L., J.S., U.R., and P.K.E.M. provided data and reviewed, revised, and approved the manuscript. U.H., B.K., A.G., J.Ä., C.B., and S.C.L. reviewed, revised, and approved the manuscript. T.F. contributed to the study design; provided data; and wrote, reviewed, revised and approved the manuscript. S.A. and T.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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