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Metabolic Profiling of Adiponectin Levels in Adults
Mendelian Randomization Analysis

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Background—Adiponectin, a circulating adipocyte-derived protein, has insulin-sensitizing, anti-inflammatory, antiatherogenic, and cardiomyocyte-protective properties in animal models. However, the systemic effects of adiponectin in humans are unknown. Our aims were to define the metabolic profile associated with higher blood adiponectin concentration and investigate whether variation in adiponectin concentration affects the systemic metabolic profile.

Methods and Results—We applied multivariable regression in ≤5909 adults and Mendelian randomization (using *cis*-acting genetic variants in the vicinity of the adiponectin gene as instrumental variables) for analyzing the causal effect of adiponectin in the metabolic profile of ≤37 545 adults. Participants were largely European from 6 longitudinal studies and 1 genome-wide association consortium. In the multivariable regression analyses, higher circulating adiponectin was associated with higher high-density lipoprotein lipids and lower very-low-density lipoprotein lipids, glucose levels, branched-chain amino acids, and inflammatory markers. However, these findings were not supported by Mendelian randomization analyses for most metabolites. Findings were consistent between sexes and after excluding high-risk groups (defined by age and occurrence of previous cardiovascular event) and 1 study with admixed population.

Conclusions—Our findings indicate that blood adiponectin concentration is more likely to be an epiphenomenon in the context of metabolicdiseasethanakeydeterminant. **(*CircCardiovascGenet*.2017;10:e001837.DOI:10.1161/CIRCGENETICS.117.001837.)**

Key Words: adiponectin ■ cardiovascular disease ■ insulin ■ metabolism ■ metabolomics

The recognition that adipose tissue is an endocrine organ raised new prospects for discovering adipose-derived products that could be valuable drug targets for the treatment and prevention of cardiometabolic diseases. In this context, adiponectin, a 30 kDa protein largely produced by mature adipocytes, has been attracting widespread attention because of insulin-sensitizing, anti-inflammatory, antiatherogenic, and cardiomyocyte-protective properties demonstrated in animal models.[1]

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However, human studies have yielded a far more complicated picture. Unlike most other adipokines, circulating adiponectin concentration is higher with lower adiposity.[2] In prospective observational studies in humans using multivariable regression, higher circulating adiponectin is associated with lower risk of type 2 diabetes mellitus,[3] hepatic dysfunction,[4] and metabolic syndrome[5] but higher mortality in patients with kidney disease, heart failure, previous cardiovascular disease, or general elderly cohorts[6–9]; this different direction of effect between risk of incident disease and mortality among high-risk groups has been called the adiponectin paradox.[10]

Given the complex metabolic derangements that might participate in and compensatory changes that might occur in response to human diseases, the association between adiponectin concentration and cardiometabolic biomarkers and disease end points might be explained by reverse causality (where disease status could alter adiponectin concentration) or residual confounding (where adiponectin could be a marker of

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another causal factor, such as adiposity or insulin resistance).[11] Classical multivariable regression studies cannot distinguish causal from noncausal associations, and randomized controlled trials specifically targeting adiponectin are not possible in the absence of a specific therapeutic targeting adiponectin concentration or function.

Mendelian randomization uses genetic variants (mostly single-nucleotide polymorphisms [SNPs]) that are robustly related to the risk factor of interest as tools to assess its role in causing disease.[12] The random allocation of parental alleles at meiosis should theoretically reduce confounding in genetic association studies, and this has been shown to be the case[13]; the unidirectional flow of biological information from genetic variant to phenotypes avoids reverse causality. Mendelian randomization has been used in clinical research to investigate potential etiologic mechanisms, such as the causal effects of low-density lipoprotein cholesterol (LDL-C),[14] systolic blood pressure,[15] and CRP (C-reactive protein)[16] on coronary heart disease, validate and prioritize novel drug targets, such as IL-6 (interleukin-6) receptor,[17] and increase understanding of current therapies, for example, statins.[18]

Previous Mendelian randomization studies indicate that circulating adiponectin is a consequence of low insulin sensitivity,[19] but whether adiponectin concentration is also a cause of insulin sensitivity is uncertain.[19–21] Using Mendelian randomization in a study of 63 746 coronary heart disease cases and 130 681 controls, we have recently shown that adiponectin may not be causally related to coronary heart disease.[22] Although multivariable analyses show that higher adiponectin concentration is associated with lower glycated hemoglobin, insulin, triglycerides [TG], and higher high-density lipoprotein cholesterol (HDL-C), using Mendelian randomization, we found little evidence that these were causal.[22] Whether adiponectin is associated with systemic metabolic profile, and, if it is, what aspects of these associations are causal is unknown. A broader interrogation of the metabolic effects of adiponectin through high-throughput profiling of metabolic status could provide valuable insights into whether adiponectin is a noncausal biomarker or causally important in the pathophysiology of some human diseases.[23]

We combined genotype, adiponectin, and metabolomics profile data from 6 longitudinal studies and 1 genome-wide association consortium with the aim of (1) defining the metabolic signature of blood adiponectin concentration and (2) investigating whether variation in adiponectin concentration is causally related to the systemic metabolic profile.

Methods

Study Populations

The metabolic profile associated with blood adiponectin concentration was examined from 7 data sources: PEL82 (the 1982 Pelotas Birth Cohort), including adults aged 30 years old born in the city of Pelotas, Brazil, in 1982[24,25]; BWHHS (the British Women's Heart and Health Study), including UK women aged 60 to 79 years old at recruitment in 2000[26]; WHII study (the Whitehall II), including UK government workers aged 45 to 69 years at phase 5 clinical assessment in 1997 to 1999[27]; the CaPS (Caerphilly Prospective Study), including men aged 52 to 72 years at phase III in 1989 to 1993[28]; a case–control study nested in UKCTOCS (the United Kingdom Collaborative Trial of Ovarian Cancer Screening), including UK postmenopausal women aged 50 to 74 years at recruitment in 2001 to 2005[29]; the ALSPAC-M (Cohort of Mothers From the Avon Longitudinal Study of Children and Parents), including UK women aged 34 to 63 years old at clinical assessment in 2009 to 2011[30]; and a metabolomics genome-wide association consortium (Metabolomics consortium), including European adults with mean age of 45 years old from 14 cohorts.[31] Individual-level data were available to investigators from PEL82, BWHHS, WHII, CaPS, UKCTOCS, and ALSPAC-M. Individual-level study data cannot be made available to other researchers for purposes of reproducing the results or replicating the procedure. Summary-level data are publicly available from the Metabolomics consortium (URL: http://www.computationalmedicine.fi/data/NMR_GWAS/).

All study participants provided written informed consent, and study protocols were approved by the local ethics committees (ethical approval for ALSPAC was also obtained from the ALSPAC Ethics and Law Committee). Studies' characteristics are summarized in Table 1. We examined (possibly causal) associations of adiponectin with systemic metabolic profiles using 2 approaches—conventional multivariable regression and Mendelian randomization analyses. Studies must have both adiponectin and measures of some of the outcomes (but do not need genetic data) to contribute to multivariable regression analyses and must have relevant genetic variants and outcomes (but do not need adiponectin concentration data) to contribute to Mendelian randomization analyses. Figure 1 shows how the different data sources contributed to the 2 approaches.

Metabolite Quantification

A high-throughput serum nuclear magnetic resonance (NMR) spectroscopy platform was used to quantify ≤150 metabolic measures and 83 derived measures (ratios) in each study. The experimental protocols, including sample preparation and NMR spectroscopy methods, have been described in detail elsewhere[32,33] and are described briefly in Methods in the Data Supplement. Sixty-six of 150 metabolic measures were selected for this study aimed at broadly representing the systemic metabolite profile, as previously reported by Würtz et al,[34] including lipoprotein traits (lipid content, particle size, and Apo [apolipoproteins]), fatty acids, amino acids, glycolysis-related metabolites, ketone bodies, fluid balance (albumin and creatinine), and inflammatory markers (glycoprotein acetyls). The remaining 84 metabolic measures from the NMR platform are related to other lipid fractions (esterified and free cholesterol, total cholesterol, TG, and phospholipids) and particle concentration from 14 lipoprotein subclasses. As these 84 metabolic measures are highly correlated with ≥1 of the 66 selected metabolic measures, they were not included in the main analysis (as they would not bring additional information) and were presented in the Data Supplement. Eight additional measures, not obtained from the NMR platform, were included: CRP, IL-6, fibrinogen, blood viscosity, insulin, glycated hemoglobin, and systolic blood pressure and diastolic blood pressure. PEL82 did not have data on metabolic measures from NMR platform and contributed data to analyses of conventional lipid risk factors (total cholesterol, HDL-C, LDL-C, and TG) and some of the additional measures described (CRP, glycated hemoglobin, systolic blood pressure, and diastolic blood pressure). Adiponectin was assayed using an ELISA in PEL82, BWHHS, and WHII. Data on adiponectin level were not available from CaPS, UKCTOCS, ALSPAC-M, and the Metabolomics consortium. Blood samples used for adiponectin, NMR metabolites, and other blood-based outcomes were taken after overnight or minimum 6-hour fast in BWHHS, CaPS, and ALSPAC-M and on nonfasting samples in PEL82 and UKCTOCS. In WHII, participants attending the morning clinic were asked to fast overnight and those attending in the afternoon were asked to have a light, fat-free breakfast before 08:00 hours. The vast majority of samples contributing to the Metabolomics consortium were fasting samples.

Genotyping

BWHHS, CaPS, WHII, and UKCTOCS participants were genotyped using Metabochip, a platform comprising 200 000 SNPs, which cover the loci identified by GWAS in cardiometabolic diseases and rare

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Table 1. Characteristics of Participating Studies

	PEL82	BWHHS	WHII	CaPS	UKCTOCS Case–Control*	ALSPAC-M	Metabolomics Consortium
Study design	Cohort	Cohort	Cohort	Cohort	Nested case–control study	Cohort	14 cohorts
Setting	Brazil	United Kingdom	United Kingdom	United Kingdom	United Kingdom	United Kingdom	Europe
Recruitment setting	Hospitals	General practices	Workplace	General practices and electoral register	Hospitals	Media information, community locations, and health services	Multiple settings
Participants	Adults aged 30 y old born in the city of Pelotas in 1982	Women aged 60–79 y old at recruitment	Civil servants aged 45–69 y at phase 5	Men aged 52–72 y old at phase III	Postmenopausal women aged 50 y old and above at recruitment	Women aged 34–63 y old residing in a defined area in the South West of England that gave birth between April 1, 1991 to December 31, 1992	Adults recruited for multiple studies (mean age: 45 y old)
Phase of data collection	2012 follow-up	Recruitment (1999–2001)	Phase 5 (1997–1999)	Phase III (1989–1993)	Recruitment (2001–2005)	Follow-up clinic assessment (2009–2011)	Data collected in different phases according to each study
Blood samples fasted	No	Yes	Mixed	Yes	No	Yes	Yes (for the vast majority of blood samples)
No. of recruited individuals at data collection phase	3701	4286	7870	2154	4867	4834	25 072
No. of recruited individuals for adiponectin	3541	498	2662	0	0	0	0
No. of recruited individuals for metabolites	3530†	3780	4641	1225	4813	4138	25 072
No. of recruited individuals for other phenotypes‡	3530–3617	3636–3964	4620–4874	608–1207	0	4092–4568	·
No. of recruited individuals for genotype	2898	1980	3078	1349	1472	8672§	25 072
No. of recruited individuals for MV analyses	2753–2762	396–497	2442–2656	·	·	·	·
No. of recruited individuals for MR analyses	2753–2783	1656–1967	2773–3020	101–1211	1067–1435	2548–3375	12 978–24 924
Website	http://www.epidemio-ufpel.org.br/site/content/coorte_1982-en/index.php	http://www.lshtm.ac.uk/eph/ncde/research/bwhhs/index.html	http://www.ucl.ac.uk/whitehallII	http://www.bristol.ac.uk/social-community-medicine/projects/caerphilly/about/	http://www.instituteforwomenshealth.ucl.ac.uk/womens-cancer/gcrc/ukctocs	http://www.bristol.ac.uk/alspac/	http://www.computationalmedicine.fi/data/NMR_GWAS/

ALSPAC-M indicates the Avon Longitudinal Study of Children and Parents-Mothers' Cohort; BWHHS, British Women's Heart and Health Study; CaPS, the Caerphilly Prospective Study; MR, Mendelian randomization; MV, multivariable; PEL82, 1982 Pelotas Birth Cohort; UKCTOCS, Case–Control Study Nested in the United Kingdom Collaborative Trial of Ovarian Cancer Screening; and WHII study, Whitehall II.

*The nested case–control study consisted of a subsample (n=4867) of the original UKCTOCS randomized controlled trial (n=202 638 recruited individuals).

†For PEL82, the only metabolites available were glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and TAG.

‡Other phenotypes include systolic and diastolic blood pressure, glycohemoglobin, C-reactive protein, interleukin-6, fibrinogen, and blood viscosity.

§DNA samples were collected for the whole cohort in prior phases of ALSPAC-M cohort.

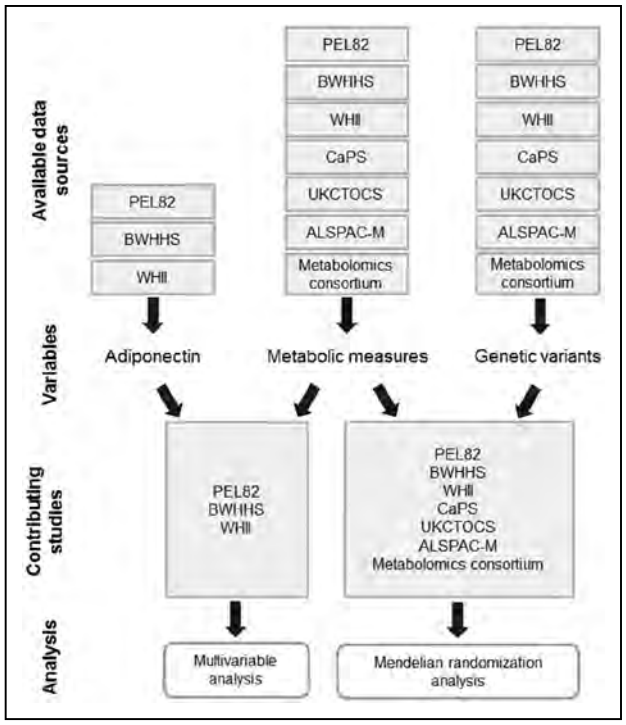

Figure 1. Schematic representation of studies contributing to each analytic approach. From the available data sources, 3 had data on adiponectin and metabolic measures and could contribute to multivariable analysis (PEL82 [1982 Pelotas Birth Cohort], BWHHS [British Women's Heart and Health Study], and WHII study [Whitehall II]), and all had data on genetic variants and metabolic measures and could contribute to Mendelian randomization analysis (PEL82, BWHHS, WHII, CaPS [the Caerphilly Prospective Study], UKCTOCS [the United Kingdom Collaborative Trial of Ovarian Cancer Screening], ALSPAC-M [Cohort of Mothers From the Avon Longitudinal Study of Children and Parents], and Metabolomics consortium).

variants from the 1000 Genomes Project.[35] Quality control criteria and imputation using 1000 Genomes European ancestry reference samples have been previously described for studies within UCL-LSHTM-Edinburgh-Bristol consortium.[36] In ALSPAC-M, 557 124 SNPs were directly genotyped using Illumina human660W quad. For quality control, SNPs were excluded if missingness >5%, Hardy–Weinberg equilibrium P value $<1\times10^{-6}$, or minor allele frequency <1%, and samples were excluded if missingness >5%, indeterminate X chromosome heterozygosity, extreme autosomal heterozygosity, or showing evidence of population stratification. Imputation was performed using 1000 genomes reference panel (Phase 1, Version 3; phased using ShapeIt v2.r644, haplotype release date December 2013) and Impute V2.2.2. For PEL82, genotyping was performed by using the Illumina HumanOmni2.5-8v1 array (Illumina Inc), and ≈2 500 000 SNPs were genotyped.[37] For PEL82, quality control criteria have been previously described,[37] and imputation was performed in 2 steps: first, genotypes were phased using SHAPEIT; then, IMPUTE2 was used for the actual imputation. For autosomal and X chromosome SNPs, 1000 Genomes Phase I integrated haplotypes (December 2013 release) and 1000 Genomes Phase I integrated variant set (March 2012 release), respectively, were used. For PEL82, ancestry-informative principal components were based on 370 539 SNPs shared by samples from the HapMap Project, the Human Genome Diversity Project, and PEL82.[38] Cohorts contributing to the Metabolomics consortium used different SNP arrays; nongenotyped SNPs were imputed using a 1000 Genomes Project March 2012 version and SNPs with accurate imputation (proper info >0.4) and minor allele count >3 were combined in fixed-effects meta-analysis using double genomic control correction. Further details can be found in the consortium publication.[31]

Other Covariates

Anthropometric variables (weight and height) were measured in each study using standard procedures, and body mass index was calculated as weight (kg)/height (m)2. Demographic and smoking status information was obtained through questionnaires.

Data Analysis

Before multivariable and genetic analyses, each study adjusted metabolic measures for age, sex, and, if applicable, place of recruitment (BWHHS and UKCTOCS) or principal components of genomic ancestry (PEL82 and some studies contributing to Metabolomics consortium), and the resulting residuals were transformed to normal distribution and standardized using inverse rank-based normal transformation. Pregnant women from PEL82 (n=73) and ALSPAC-M (n=12) were excluded. As the 74 analyzed metabolic measures are highly correlated, we adopted a similar strategy to the Metabolomics consortium[31] to correct for multiple testing by estimating the number of independent tests as the number of principal components that explained over 95% of variance in metabolic measures using data from the 2 studies (BWHHS and WHII) with the largest available number of metabolites (n=27 principal components in both studies). As a result, for both multivariable and Mendelian randomization analyses, we corrected for multiple testing using the Bonferroni method considering 27 independent tests (P=0.05/27≈0.0019). Analyses were conducted in Stata version 12.

Multivariable Regression Analysis

The conventional multivariable regression association of adiponectin with individual metabolites was estimated using a 2-stage individual participant meta-analysis. In the first stage, linear regression models were fitted for each study. In the second stage, study-specific estimates were meta-analyzed using DerSimonian and Laird random-effect model.[39] Heterogeneity across studies was assessed using I^2 (as a measure of the relative size of between-study variation and within-study error).[40] Three types of subgroup analyses were conducted: sex-stratified analysis, analysis excluding individuals with high risk of cardiometabolic disease (those that had experienced coronary artery disease or stroke or those older than 65 years), and analysis restricted to European studies (excluding PEL82).

Genetic Analyses

Four independent SNPs in the vicinity of *ADIPOQ* locus (±50 kb), previously identified to predict adiponectin levels, were selected[22,41] (details in Methods in the Data Supplement). These SNPs (rs6810075, rs16861209, rs17366568, and rs3774261) are estimated to explain ≈4% of variance in adiponectin concentration (details in Methods in the Data Supplement). Data for the association of each selected SNP with adiponectin concentration in the discovered GWAS sample were downloaded from ADIPOGen website (https://www.mcgill.ca/genepi/adipogen-consortium).

Association of Genetic Variants With Classical Confounders

The association between genetic variants and classical confounders (sex, age, ancestry [European versus non-European], current smoking [yes versus no], and body mass index) was examined for each study that provided individual-level data using logistic or linear regression models for binary or continuous variables, respectively.

Mendelian Randomization Analysis

To allow all participants with relevant genetic and metabolic measure data to contribute to analyses, even when adiponectin data were not available (as in CaPS, UKCTOCS, ALSPAC-M, and Metabolomics consortium), a 2-sample Mendelian randomization design was used, in which data for the association between genetic variants and adiponectin levels were obtained from an external data source, the ADIPOGen consortium.[42] The 2-sample Mendelian randomization is a recent extension to the more conventional 1-sample Mendelian randomization

and has the additional advantage of avoiding bias because of genetic variants correlating with confounders by chance (statistical overfitting) when samples are independent.[43] The 2-sample Mendelian randomization estimates and respective SEs were obtained by combining SNP-specific Wald ratios, as described by Burgess et al[44] and detailed in Methods in the Data Supplement. Study-specific Mendelian randomization estimates were meta-analyzed using DerSimonian and Laird random-effect model.[39] Heterogeneity across studies was assessed using I^2.[40] Subgroup analyses were conducted considering individual-level (sex and risk of cardiometabolic disease) and study-level characteristics (European versus non-European studies). The Metabolomics consortium did not contribute to subgroup analysis of individual-level characteristics as only summary data were available.

Comparison Between Multivariable and Mendelian Randomization Analyses

Results from conventional multivariable and Mendelian randomization analyses for each metabolic measure were compared using the Z test (details in the Methods in the Data Supplement) and by estimating the correlation between multivariable and Mendelian randomization estimates across all metabolic measures. Power calculations for multivariable and Mendelian randomization analysis are available in Table I in the Data Supplement.

Results

The study included a median sample size of 3008 adults in the multivariable analysis (range: 2470–5909) and a median sample size of 29 146 adults in the Mendelian randomization analysis (range: 4647–37 545). Total sample size for each metabolite in multivariable and Mendelian randomization analysis can be found in Table II in the Data Supplement. Characteristics of participants and distribution of metabolites from each contributing study are listed in Table 2 and Table III in the Data Supplement.

Adiponectin and the Systemic Metabolic Profile

In the multivariable analysis, adiponectin was associated with 59 of 74 (80%) metabolites at nominal level ($P<0.05$) and 49 of 74 (66%) after correcting for multiple testing ($P<0.0019$). Overall, higher circulating adiponectin was associated with a healthier systemic metabolite profile. Blood adiponectin concentration was strongly related to multiple lipoprotein traits. With higher adiponectin concentration, lipid concentration was lower in very LDL subclasses and higher in HDL subclasses, except for small HDL. There was no strong evidence of circulating adiponectin associating with total lipid content in LDL subclasses or in intermediate-density lipoprotein, although adiponectin concentration was inversely associated with LDL-TG. Higher adiponectin was associated with lower concentration of cholesterol, TG, and lower mean particle diameter in very LDL, as well as higher cholesterol concentration and mean particle diameter in HDL. Higher adiponectin concentration was also associated with higher concentration of Apo AI and phospholipids and lower concentration of TG and diglycerides (Figure 2).

Higher circulating adiponectin was also associated with healthier glycemic status (lower glucose and insulin concentration), lower blood concentration of glycolysis-related metabolites (lactate and pyruvate), saturated fatty acids, systemic inflammatory markers (CRP, fibrinogen, IL-6, glycoprotein

Table 2. Characteristics of Studies' Populations

	PEL82	BWHHS	WHII	CaPS	UKCTOCS	ALSPAC-M	Metabolomics Consortium
%							
Male	49	0	72	100	0	0	45
White	75	100	93	100	97	97	NA*
Smoker	24	12	17	20	…	11	NA
Overweight/obese	58	72	57	69	60	56	NA
Median (p25, p75)							
Age, y	30 (30, 30)	69 (64, 73)	55 (51, 61)	56 (53, 60)	66 (60, 70)	48 (45, 51)	45 (24, 61)†
Adiponectin, µg/mL	7.9 (5.2, 11.9)	15.8 (10.8, 21.5)	8.5 (6.1, 12)	…	…	…	…
Glucose, mmol/L	4.8 (4.4, 5.3)	4.7 (4.3, 5.1)	5 (4.7, 5.4)	3.8 (3.5, 4.2)	2.2 (1.7, 3.1)	4.4 (4.1, 4.7)	NA
HDL-C, mmol/L	1.5 (1.2, 1.7)	1.6 (1.4, 1.9)	1.5 (1.3, 1.7)	0.9 (0.7, 1)	1.6 (1.4, 1.9)	1.7 (1.5, 1.9)	NA
LDL-C, mmol/L	2.7 (2.3, 3.3)	2.3 (1.9, 2.8)	1.9 (1.6, 2.2)	1.6 (1.3, 1.9)	1.8 (1.4, 2.2)	1.5 (1.2, 1.8)	NA
TG, mmol/L	1.1 (0.8, 1.6)	1.5 (1.1, 2)	1.1 (0.9, 1.5)	1.5 (1.2, 2)	1.5 (1.1, 2.1)	0.9 (0.7, 1.2)	…
SBP, mm Hg	120 (112, 130)	146 (130, 163)	121 (111, 133)	144 (130, 160)	…	117 (110, 125)	…
DBP, mm Hg	75 (69, 81)	79 (71, 87)	77 (70, 84)	84 (76, 92)	…	71 (66, 77)	…

ALSPAC-M indicates the Avon Longitudinal Study of Children and Parents–Mothers' Cohort; BWHHS, British Women's Heart and Health Study; CaPS, the Caerphilly Prospective Study; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NA: not available; PEL82, 1982 Pelotas Birth Cohort; SBP, systolic blood pressure; TG, triglycerides; UKCTOCS, Case–Control Study Nested in the United Kingdom Collaborative Trial of Ovarian Cancer Screening; and WHII study, Whitehall II.

*Cohorts contributing to the Metabolomics consortium were of European origin.

†Overall mean age (and range of mean age across studies).

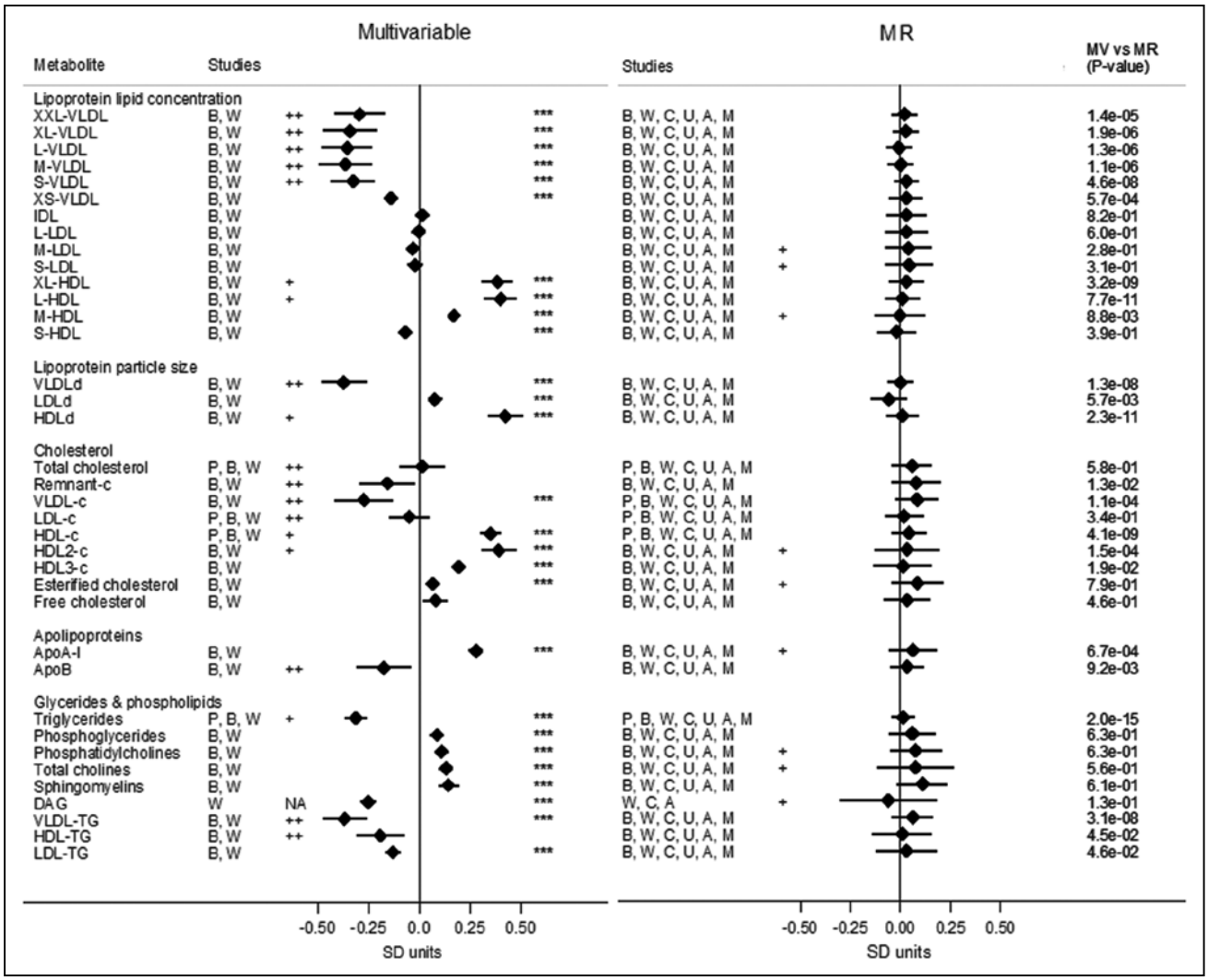

Figure 2. Association of lipoprotein traits with blood adiponectin levels from observational and Mendelian randomization (MR) analysis. Values are expressed as units of standardized log metabolite concentration (and 95% CI [confidence interval]) per 1 U increment of standardized log adiponectin levels. *P* values for the association between adiponectin and metabolites are indicated by *** if lower than Bonferroni-adjusted threshold (*P* value <0.0019). Heterogeneity was considered substantial if I^2=50% to 75% (+) or high if I^2>75% (++). *P* values for the comparison between multivariable and MR estimates are displayed in the column MR vs MV (*P* value). Metabolic measures were adjusted for age, sex, and, if applicable, place of recruitment (BWHHS [British Women's Heart and Health Study] and UKCTOCS [the United Kingdom Collaborative Trial of Ovarian Cancer Screening]) or principal components of genomic ancestry (PEL82 [1982 Pelotas Birth Cohort] and some studies contributing to Metabolomics consortium), and the resulting residuals were transformed to normal distribution by inverse rank-based normal transformation. A indicates the Avon Longitudinal Study of Children and Parents-Mothers' Cohort; Apo, apolipoprotein; B, BWHHS; C, the Caerphilly Prospective Study; DAG, diglycerides; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; IDL, intermediate-density lipoprotein; L, large; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; M, medium; M, Metabolomics consortium; P, PEL82; S, small; TG, triglycerides; U, UKCTOCS Nested Case–Control Study; VLDL, very-low-density lipoprotein; VLDL-C, VLDL cholesterol; W, Whitehall II Study; XL, very large; XS, very small; and XXL: extremely large.

acetyls, and blood viscosity), blood pressure, creatinine, and higher ketone bodies (acetoacetate). In addition, higher adiponectin concentration was associated with lower concentrations of free branched-chain amino acids (isoleucine, leucine, and valine), aromatic amino acids (phenylalanine and tyrosine), and alanine and higher concentration of glutamine (Figure 3).

In the multivariable analyses, evidence of heterogeneity in pooled estimates across studies was substantial (I^2=50%–75%) for 12 and high (I^2>75%) for 15 metabolic measures (Figures 2 and 3; Tables IVA and V in the Data Supplement). This did not seem to be accounted by sex (Figures I through IV in the Data Supplement), geographic location (Figures V and VI in the Data Supplement), or high risk of disease (Figures VII and VIII in the Data Supplement). Results were consistent for metabolites not included in the main analysis (Figures IX and X in the Data Supplement).

Causal Effects of Adiponectin on the Systemic Metabolic Profile

Characteristics of the 4 SNPs (rs6810075, rs16861209, rs17366568, and rs3774261) used in Mendelian randomization and their association with adiponectin concentration are shown in Table 3. Overall, SNPs effect allele frequency was similar across studies. Two SNPs had lower allele frequency in the Metabolomics consortium (rs6810075: 51% versus 65%–69% in other studies; rs16861209: 5% versus 9%–11%

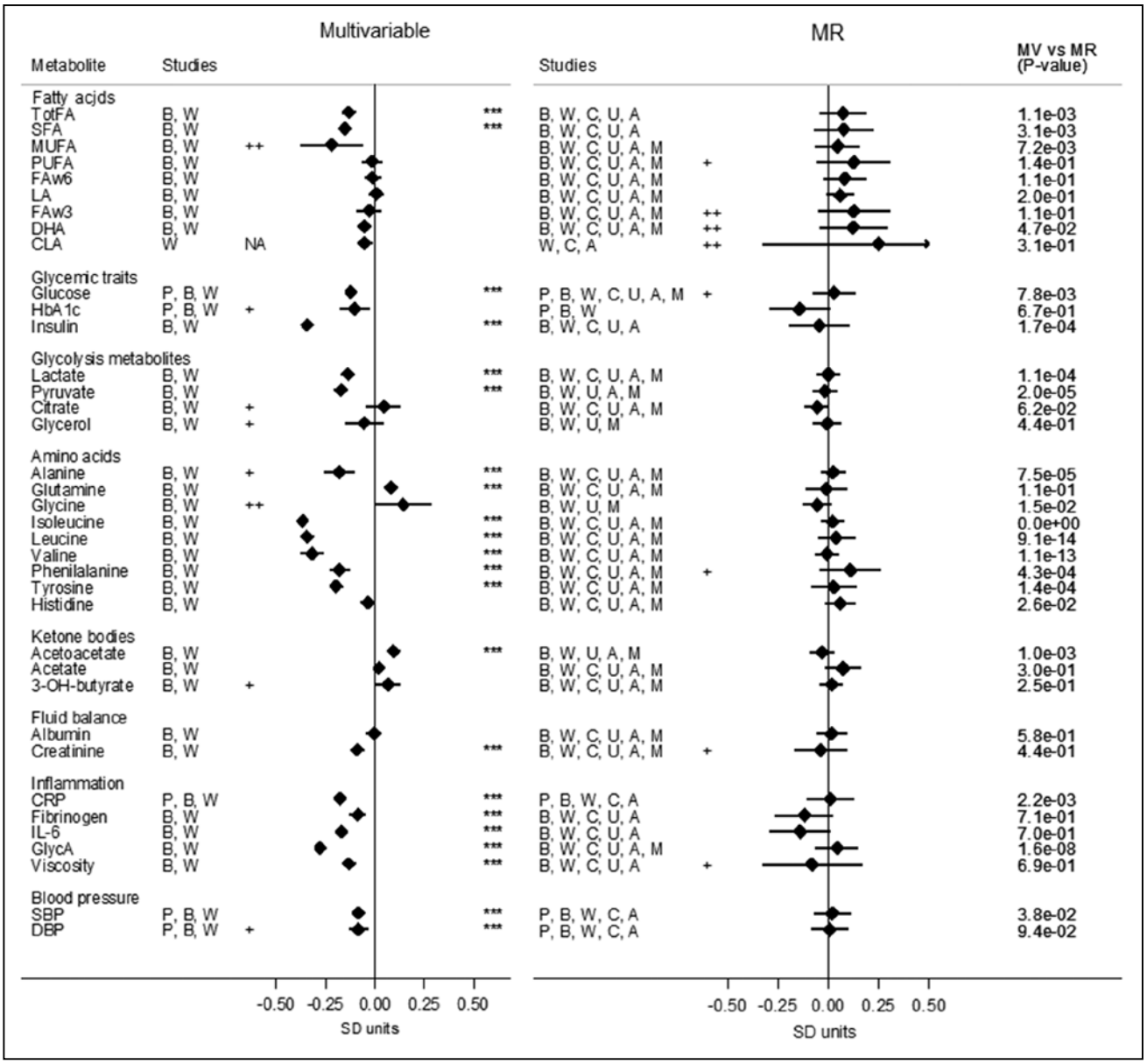

Figure 3. Association of multiple metabolic measures with blood adiponectin levels from observational and Mendelian randomization (MR) analysis. Values are expressed as units of standardized log metabolite concentration (and 95% CI [confidence interval]) per 1 U increment of standardized log adiponectin levels. *P* values for the association between adiponectin and metabolites are indicated by *** if lower than Bonferroni-adjusted threshold (*P* value <0.0019). Heterogeneity was considered substantial if I^2=50% to 75% (+) or high if I^2>75% (++). *P* values for the comparison between multivariable and MR estimates are displayed in the column MR vs MV (*P* value). Metabolic measures were adjusted for age, sex, and, if applicable, place of recruitment (BWHHS [British Women's Heart and Health Study] and UKCTOCS [the United Kingdom Collaborative Trial of Ovarian Cancer Screening]) or principal components of genomic ancestry (PEL82 [1982 Pelotas Birth Cohort] and some studies contributing to Metabolomics consortium) and the resulting residuals were transformed to normal distribution by inverse rank-based normal transformation. A, the Avon Longitudinal Study of Children and Parents-Mothers' Cohort; B, BWHHS; C, the Caerphilly Prospective Study; CLA, conjugated linoleic acids; CRP, C-reactive protein; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; FAw3, omega-3 fatty acid; FAw6, omega-6 fatty acid; GlycA, glycoprotein acetyls; HbA1c, glycated hemoglobin; IL-6, interleukin-6; LA, linoleic acid; M, Metabolomics consortium; MUFA, monounsaturated fatty acid; P, PEL82; PUFA: polyunsaturated fatty acids; SBP, systolic blood pressure; SFA, saturated fatty acid; TotFA: total fatty acids; U, UKCTOCS Nested Case–Control Study, and W, Whitehall II Study.

in other studies), and 1 SNP had a higher frequency in PEL82 compared with other studies (rs3774261: 49% versus 38%–39% in other studies; Table 3). As expected, the selected SNPs were not associated with classical confounders overall (Table VI in the Data Supplement).

Findings from Mendelian randomization analysis were largely inconsistent with results from multivariable analysis. First, there was no evidence that adiponectin influenced HDL and very LDL traits (Figure 2). Second, genetically increased adiponectin levels were not associated with glycemic traits, free amino acids, and glycolysis-related metabolites (Figure 3). Results were less conclusive for some inflammatory markers (IL-6 and fibrinogen; Figure 3). Third, there was strong statistical evidence that associations from multivariable

Table 3. Characteristics of SNPs Selected for Mendelian Randomization Analysis

	SNP			
	rs6810075	rs16861209	rs17366568	rs3774261
Chr	3	3	3	3
Position*	186548565	186563114	186570453	186571559
Closest gene	ADIPOQ	ADIPOQ	ADIPOQ-AS1, ADIPOQ	ADIPOQ-AS1, ADIPOQ
EA	T	A	G	A
NEA	C	C	A	G
ADIPOGen consortium				
EAF†	0.63	0.07	0.90	0.39
β‡	0.11	0.31	0.25	0.11
SE	0.01	0.02	0.01	0.01
PEL82				
EAF	0.65	0.11	0.92	0.49
β	0.13	0.33	0.22	0.08
SE	0.03	0.04	0.05	0.03
R^2	0.008	0.021	0.005	0.002
BWHHS				
EAF	0.67	0.09	0.89	0.38
β	0.32	0.30	1.04	0.30
SE	0.10	0.14	0.24	0.08
R^2	0.022	0.020	0.051	0.044
WHII				
EAF	0.68	0.10	0.89	0.38
β	0.16	0.36	0.56	0.14
SE	0.04	0.05	0.08	0.03
R^2	0.008	0.027	0.025	0.010
CaPS§				
EAF	0.69	0.10	0.89	0.39
UKCTOCS§				
EAF	0.69	0.10	0.89	0.38
ALSPAC-M§				
EAF	0.66	0.09	0.93	0.38
Metabolomics consortium§				
EAF	0.51	0.05	0.88	0.36

β (and SE) refers to mean difference in standardized log adiponectin per additional SNP effect allele. ALSPAC-M indicates the Avon Longitudinal Study of Children and Parents-Mothers' Cohort; BWHHS, British Women's Heart and Health Study; CaPS, the Caerphilly Prospective Study; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; NEA, noneffect allele; PEL82, 1982 Pelotas Birth Cohort; SNP, single-nucleotide polymorphism; UKCTOCS, case–control study nested in the United Kingdom Collaborative Trial of Ovarian Cancer Screening; and WHII study, Whitehall II.

*Genome Reference Consortium Human Build 37. For CaPS, UKCTOCS, ALSPAC-M, and the Metabolomics consortium, data on adiponectin levels were not available.

†Extracted from Dastani et al (2012).[42]

and Mendelian randomization analyses were inconsistent with each other (Figures 2 and 3), and the overall correlation between multivariable and Mendelian randomization estimates was low (r=0.10; Figure 4). Finally, in the Mendelian randomization analysis, adiponectin was not associated with any of the metabolic analyses at either P <0.05 or P <0.0019.

In the Mendelian randomization analyses, evidence of heterogeneity in pooled estimates across studies was substantial (I^2=50%–75%) for 14 and high (I^2>75%) for 3 metabolic measures, suggesting lower heterogeneity in models from genetic analysis than from the multivariable analyses (Figures 2 and 3; Tables IVB and V in the Data Supplement). This did not seem to be driven by sex differences (Figures I through IV in the Data Supplement), geographic location/ethnicity (Figures V and VI in the Data Supplement), or high risk of disease (Figures VII and VIII in the Data Supplement). Results were consistent with no association between adiponectin and metabolites not included in the main analysis (Figures IX and X in the Data Supplement).

Discussion

In ≤5909 adults, we found using multivariable regression analyses that circulating adiponectin was associated with a pattern of systemic metabolites levels associated with good health. Higher blood adiponectin concentration was associated with higher HDL lipids and lower very LDL lipids, glycemia, and branched-chain amino acids levels. However, when we used genetic variants in the vicinity of adiponectin-encoding gene to test the causal effect of adiponectin on systemic metabolic profiles among ≤37 545 adults, we found little evidence that the associations were causal.

Genetic association studies indicate that genetic variants associated with circulating adiponectin (in loci other than *ADIPOQ*) are also associated with cardiometabolic outcomes, such as type 2 diabetes mellitus[42] and coronary heart disease[41]; however, this is likely to be reflecting a pleiotropic effect of these variants. Our findings and previous Mendelian randomization studies[19,22] suggest that the association between circulating adiponectin and metabolic biomarkers and cardiometabolic diseases is likely to be explained by shared factors (confounding) rather than by a direct role of adiponectin on metabolism and downstream cardiometabolic disease. These results are in contrast to findings from animal models pointing to insulin-sensitizing and antiatherogenic actions of adiponectin.[1]

Circulating adiponectin is known to be substantially reduced among obese individuals, particularly in the presence of central fat accumulation.[45] A recent Mendelian randomization study examining the causal metabolic effects of body mass index demonstrated that lower body mass index was related to favorable lipoprotein subclass profile and lower concentration of branched-chain amino acids, inflammatory markers, and insulin,[34] which is remarkably similar to our results from the conventional multivariable analysis. In addition, numerous studies have shown that adiponectin production is suppressed by insulin action in humans, which seems to be at least partly attributed to regulation at the transcriptional level.[46] As an example, elevated circulating adiponectin is found in contexts of both primary deficiency of insulin (type

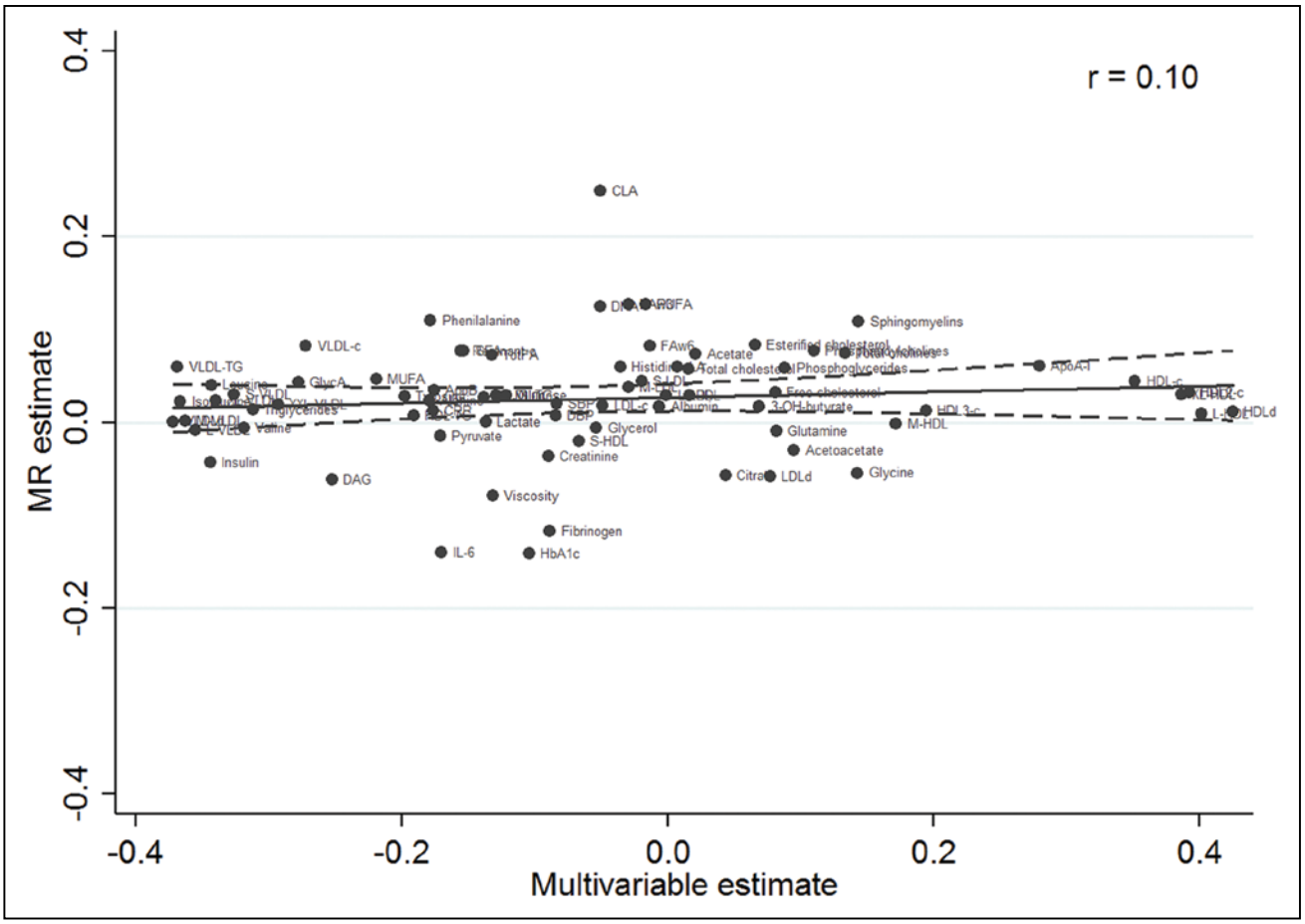

Figure 4. Correlation between estimates from multivariable regression and Mendelian randomization (MR). Apo indicates apolipoprotein; CLA, conjugated linoleic acids, CRP, C-reactive protein; DAG, diglycerides; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; FAw3, omega-3 fatty acid; FAw6, omega-6 fatty acid; GlycA, glycoprotein acetyls; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; IDL, intermediate-density lipoprotein; IDL-C, IDL cholesterol; IL-6, interleukin-6; L, large; LA, linoleic acid; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; M, medium; MUFA, monounsaturated fatty acid; *r*, Pearson correlation coefficient; S, small; SBP, systolic blood pressure; SFA, saturated fatty acid; TG, triglycerides; VLDL, very-low-density lipoprotein; VLDL-C, VLDL cholesterol; XL, very large; XS, very small; and XXL, extremely large.

1 diabetes mellitus)[47] and global insulin resistance because of genetic or acquired defects in the insulin receptor.[48] Genetic predisposition to insulin resistance and central fat accumulation[45,49] is related to lower circulating adiponectin. Evidence from animal models has raised the possibility of a bidirectional relationship between adiponectin and insulin concentration.[50] Early Mendelian randomization studies did indicate that adiponectin could mitigate insulin resistance[20,21]; however, these results could not be replicated in a larger Mendelian randomization study,[19] as well as in our study presented here. The well-known metabolic effects of adiposity and insulin on circulating adiponectin concentration reinforce that the clustering of adiponectin and several traditional and novel biomarkers is likely to result from confounding because of increasing adiposity and disruption of insulin action.

Strengths of our study include detailed metabolic profile in several longitudinal studies, which enabled us to characterize the metabolic profile of high adiponectin concentration beyond traditional biomarkers, as well as the use of Mendelian randomization to disentangle the causal effect of adiponectin on the metabolism. Mendelian randomization analysis can reliably test for the presence of a causal relation under the 3 assumptions of an instrumental variable that the genetic variants (1) are robustly associated with the risk factor of interest (adiponectin), (2) should only affect the outcome (metabolites) through the exposure, and (3) are not associated with exposure–outcome confounders.[51] To ensure that IV assumptions were met, or were at least plausible, we only used SNPs strongly and specifically (within *ADIPOQ* gene) related to adiponectin concentration as instrumental variables and we adjusted for population structure in models using data from PEL82 to avoid confounding by population stratification. One of the limitations of our study was the limited power in subgroup analyses including only individual-level data (sex- and risk-stratified analyses), which limited our investigation of potential sources of heterogeneity. Another limitation was the absence of data on high–molecular weight adiponectin, which is believed to account for most of the adiponectin biological effects in experimental settings. However, most human (and many animal model) studies have not used high–molecular weight adiponectin, and we found the same multivariable observational associations as in previous

studies. Also, it should be emphasized that SNPs in *ADIPOQ* gene are associated with both total and high–molecular weight adiponectin,[52–54] including SNPs we used in our analysis (eg, rs3774261)[52] or others in high linkage disequilibrium with these (eg, rs17300539–R^2 >0.8 with rs16861209).[53,54]

Overall, our findings suggest that low circulating adiponectin is likely to reflect adipocyte dysfunction and that altered total blood adiponectin concentration is an epiphenomenon in the context of metabolic disease, rather than a key determinant. Therefore, interventions targeting manipulation of adiponectin concentration are unlikely to result in therapeutic benefits for tackling cardiometabolic diseases. Our results highlight the potential of Mendelian randomization analysis and high-throughput metabolomics profiling to yield important insights to advance our understanding in the pathophysiology of common complex diseases and to inform which targets are best bets for taking forward into drug development, given that drug target validation is a key obstacle underlying the unsustainably high rate of drug development failure. Although our and other studies suggest that adiponectin is not a valuable target for developing drugs aimed at preventing cardiometabolic diseases, it may nonetheless be a valuable biomarker for predicting these diseases given the wide-ranging associations shown here. The associations we have found would need to be replicated in additional independent studies before testing their ability to predict disease outcomes.

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Disclosures

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CLINICAL PERSPECTIVE

Adiponectin, a protein produced by adipose cells, has insulin-sensitizing, anti-inflammatory, antiatherogenic, and cardiomyocyte-protective properties in animal models. In prospective studies in humans, higher circulating adiponectin is associated with lower risk of type 2 diabetes mellitus, hepatic dysfunction, and metabolic syndrome. However, it is not clear whether adiponectin is protective against these metabolic disorders or whether these associations are just reflecting reverse causality (where disease status could alter adiponectin concentration) or residual confounding (where adiponectin could be a marker of another causal factor, such as adiposity or insulin resistance). We used Mendelian randomization to clarify whether circulating adiponectin is causally related to the metabolic profile of ≤37 545 adults. Four common genetic variants nearby the gene encoding adiponectin (*ADIPOQ*) were used as instruments to test the effect of circulating adiponectin on 74 metabolic measures selected to broadly represent the systemic metabolite profile, including lipoprotein subclasses, fatty acids, glycemic traits, free amino acids, inflammatory markers, and blood pressure. Overall, our findings do not support a direct role of circulating adiponectin on the systemic metabolic profile in humans and indicate that the clustering of adiponectin and several traditional and novel biomarkers is likely to result from confounding or reverse causality. Therefore, interventions targeting manipulation of adiponectin concentration are unlikely to result in therapeutic benefits for tackling metabolic diseases.

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