# Mendelian Randomization Studies Do Not Support a Causal Role for Reduced Circulating Adiponectin Levels in Insulin Resistance and Type 2 Diabetes

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Adiponectin is strongly inversely associated with insulin resistance and type 2 diabetes, but its causal role remains controversial. We used a Mendelian randomization approach to test the hypothesis that adiponectin causally influences insulin resistance and type 2 diabetes. We used genetic variants at the *ADIPOQ* gene as instruments to calculate a regression slope between adiponectin levels and metabolic traits (up to 31,000 individuals) and a combination of instrumental variables and summary statistics–based genetic risk scores to test the associations with gold-standard measures of insulin sensitivity (2,969 individuals) and type 2 diabetes (15,960 case subjects and 64,731 control subjects). In conventional regression analyses, a 1-SD decrease in adiponectin levels was correlated with a 0.31-SD (95% CI 0.26–0.35) increase in fasting insulin, a 0.34-SD (0.30–0.38) decrease in insulin sensitivity, and a type 2 diabetes odds ratio (OR) of 1.75 (1.47–2.13). The instrumental variable analysis revealed no evidence of a causal association between genetically lower circulating adiponectin and higher fasting insulin (0.02 SD; 95% CI −0.07 to 0.11; $N$ = 29,771), nominal evidence of a causal relationship with lower insulin sensitivity (−0.20 SD; 95% CI −0.38 to −0.02; $N$ = 1,860), and no evidence of a relationship with type 2 diabetes (OR 0.94; 95% CI 0.75–1.19; $N$ = 2,777 case subjects and 13,011 control subjects). Using the *ADIPOQ* summary statistics genetic risk scores, we found no evidence of an association between adiponectin-lowering alleles and insulin sensitivity (effect per weighted adiponectin-lowering allele: −0.03 SD; 95% CI −0.07 to 0.01; $N$ = 2,969) or type 2 diabetes (OR per weighted adiponectin-lowering allele: 0.99; 95% CI 0.95–1.04; 15,960 case subjects vs. 64,731 control subjects). These results do not provide any consistent evidence that interventions aimed at increasing adiponectin levels will improve insulin sensitivity or risk of type 2 diabetes. ***Diabetes*** **62:3589–3598, 2013**

Circulating adiponectin levels are strongly inversely correlated with insulin resistance and risk of type 2 diabetes (1,2), but the causal directions of these associations are unclear. The correlation between fasting insulin and circulating adiponectin levels is between ~0.3 and 0.4, a correlation of about half of that between fasting insulin and BMI. Adiponectin is also inversely correlated with BMI, and its association with insulin resistance might be confounded by BMI. There are some studies that suggest that the association between adiponectin and insulin remains as strong, or even stronger, when correcting for BMI (3–5). The strength of the association has led to suggestions that adiponectin could be used as a putative insulin-sensitizing treatment (6–8). Evidence

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from genetically or pharmacologically manipulated murine models suggests lowering adiponectin could induce insulin resistance. These studies were usually conducted using models challenged by a metabolic stressor such as high-fat feeding or lipodystrophy (8,50–55). Evidence from human studies is less clear (9,40–42,45–48,56–58) but includes data from a recent genome-wide association study (GWAS) that showed an association between an adiponectin genetic risk score and fasting insulin and type 2 diabetes (12) and a recent Mendelian randomization study using 942 individuals that suggested a causal role for adiponectin in insulin sensitivity (10).

In this study, we used the principle of Mendelian randomization (11) to investigate the causal nature of the association among circulating adiponectin levels, insulin resistance, type 2 diabetes, and related metabolic traits. We used a combination of four genetic variants within the adiponectin-encoding gene *ADIPOQ* that explain 4% of the variance in circulating adiponectin levels and up to 31,000 individuals with adiponectin, genetic variants, and metabolic trait outcomes measured. In contrast to previous studies that have used genetic variants to examine causation in this relationship (10,12,13), our analyses used an instrumental variables approach, limited genetic variants to those in the *ADIPOQ* gene (providing a test very unlikely to be influenced by pleiotropy), and performed the analyses using tens of thousands of individuals with both circulating adiponectin and fasting insulin measurements.

## RESEARCH DESIGN AND METHODS

**Study design.** We used two study designs (Supplementary Fig. 1). In the first design, we used an instrumental variables approach. We used studies in which adiponectin had been measured as well as fasting insulin or type 2 diabetes status (our two primary outcomes) and other related metabolic traits (fasting glucose, BMI, triglycerides, HDL cholesterol [HDL-C], LDL cholesterol [LDL-C], and total cholesterol). We used up to 31,000 individuals of European descent from 13 studies (Table 1 and Supplementary Table 1) and up to 5,100 individuals of non-European descent from 3 studies (Supplementary Table 2). These data included 1,860 individuals from 3 studies with single nucleotide polymorphisms (SNPs), adiponectin, and a measure of insulin sensitivity, including the previously published Uppsala Longitudinal Study of Adult Men (ULSAM) (10), Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC), and Minnesota study.

In the second study design, we used an adiponectin summary statistics genetic risk score, in which measured adiponectin levels were not required. For type 2 diabetes, we used a total of 15,960 diabetic case subjects and 64,731 control subjects (including results for three available adiponectin SNPs from the DIAbetes Genetics Replication And Meta-analysis [DIAGRAM] [8,130 case subjects vs. 38,987 control subjects]) (14) and results from seven studies not in the DIAGRAM (7,830 case subjects vs. 25,744 control subjects; Supplementary Tables 1 and 3). For insulin sensitivity, we used a meta-analysis of M-value and insulin suppression test GWAS results from the GENESIS consortium (RISC, ULSAM, Eugene2, Stanford; Supplementary Table 4) and the Minnesota study (Supplementary Table 1) consisting of 2,969 individuals of European descent.

**Selection of SNPs.** We limited our selection of genetic variants to those in or near *ADIPOQ*, the gene that encodes the adiponectin protein. This approach meant that our genetic instrument was less likely to violate the Mendelian randomization assumption that the instrument should only affect the outcome through the exposure of interest. We selected a set of SNPs (rs17366653, rs17300539, rs3774261, and rs3821799) that explained 4% of the variance in adiponectin levels. Details of genotyping and quality control are given in Supplementary Table 1.

**Exposure and outcome variables.** Details of adiponectin measures (exposure of interest) are given in Supplementary Table 1. Our primary outcomes were fasting insulin (as a proxy of insulin resistance) and type 2 diabetes. Our secondary outcomes were insulin sensitivity (M-value or insulin suppression test), fasting glucose, HDL-C, LDL-C, BMI, triglycerides, and total cholesterol (Supplementary Table 1).

For each European study, individuals of non-European descent were removed. For the analyses of continuous metabolic outcomes (fasting insulin, fasting glucose, HDL-C, LDL-C, BMI, glucose, triglycerides, and total cholesterol) we excluded: *1*) individuals with type 2 diabetes; *2*) individuals with fasting glucose values ≥7.0 mmol/L and/or 2-h oral glucose tolerance test

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TABLE 1
Summary details and relevant characteristics of European studies

| Study | *N* (males/females) | Age (years) | BMI | Adiponectin (μg/mL) | Fasting plasma insulin (pmol/L) |
|---|---|---|---|---|---|
| BWHHS | 3,904 (0/3,904) | 68.83 (5.5) | NA | 16.04 (7.6) | 9.46 (24.7) |
| CoLaus | 6,152 (2,922/3,230) | 53.00 (11.0) | 25.80 (4.6) | 9.90 (8.1) | 52.80 (37.2) |
| ELY | 1,570 (731/839) | 53.27 (7.7) | 25.65 (3.8) | 7.62 (3.8) | 44.92 (31.9) |
| ERF | 2,812 (1,248/1,564) | 49.76 (15.0) | 26.84 (4.6) | 10.52 (5.6) | 13.25 (7.5) |
| Fenland | 4,338 (2,010/2,328) | 46.15 (7.2) | 26.83 (4.9) | 5.80 (2.2) | 35.90 (14.8) |
| FHS | 4,488 (2,064/2,424) | 49.26 (9.3) | 27.32 (5.2) | 9.38 (5.9) | 24.33 (18.4) |
| GoDARTS | 3,696 (1,842/1,854) | 62.57 (12.1) | 27.50 (4.6) | 4.96 (3.9) | 36.00 (27.6) |
| METSIM | 8,156 (8,156/0) | 57.50 (7.0) | 26.80 (3.8) | 6.90 (4.1) | 38.40 (34.2) |
| Minnesota | 221 (116/105) | 21.13 (2.7) | 24.17 (3.7) | 8.62 (3.5) | 49.00 (20.5) |
| RISC | 1,031 (453/578) | 43.96 (8.4) | 25.47 (4.0) | 8.34 (3.7) | 34.40 (18.7) |
| SAPHIR | 1,770 (1,107/663) | 51.39 (6.0) | 25.99 (5.1) | 7.60 (5.3) | 36.00 (27.5) |
| TUK | 1,399 (0/1,399) | 48.10 (11.3) | 25.43 (4.9) | 7.99 (3.7) | 12.60 (16.1) |
| YF | 1,844 (825/1,019) | 39.00 (5.0) | 25.13 (5.5) | 9.04 (6.8) | 47.99 (42.8) |

Data are mean (SD) unless otherwise indicated. NA, not applicable.

glucose ≥11.1 mmol/L. For the analyses of type 2 diabetes, we excluded: in case subjects, *1*) individuals aged at diagnosis <35 or >70 years; *2*) individuals who needed insulin treatment within 1 year of diagnosis; and *3*) individuals aged <45 years whose age at diagnosis was not known at the time of study; and in control subjects, *1*) individuals aged <35 or >70 years at the time of study; and *2*) individuals with $HbA_{1c}$ >6.4% and/or fasting glucose >7 mmol/L.

Continuous variables (Supplementary Table 1) that were not normally distributed were $\log_{10}$-transformed. We then took the residuals of the standard linear regression using two covariates, age and sex, and, if applicable to the study, principle components, center, or other measures required to correct for ethnicity. We inverse-normal transformed all variable levels in each individual study to enable meta-analyses.

**SNP–trait association.** We performed SNP–trait associations in each study using two different models: *1*) a univariable model in which each SNP was analyzed separately; and *2*) a multivariable model in which all four SNPs were used together. The multivariable model accounts for correlation between the SNPs due to linkage disequilibrium. We used an additive genetic model.

**Instrumental variable analysis.** To estimate the causal effect of adiponectin levels on metabolic outcomes, we performed instrumental variable analyses using the four *ADIPOQ* SNPs entered separately into the same model (11). We applied the two-stage least-squares estimator method that uses predicted levels of adiponectin per genotype and regresses each outcome against these predicted values.

For continuous metabolic outcomes, we performed all of the instrumental variable analyses either in Stata using the ivreg2 command or in R using the tsls command from library (sem). The Framingham Heart Study (FHS) used a two-stage approach (similar to the approach used for type 2 diabetes, please see the following) to correct for familial correlation. For type 2 diabetes, we performed instrumental variable analysis in two stages. First, we assessed the association between the four SNPs and inverse-normal transformed adiponectin levels. We saved the predicted values and residuals from this regression model. Second, we used the predicted values from stage 1 as the independent variable (reflecting an unconfounded estimate of adiponectin levels) and diabetes status as the dependent variable in a logistic regression analysis. Both stages were performed either in R or Stata. We examined *F*-statistics from first-stage regressions to evaluate the strength of the instruments; weak instruments can bias results toward the (confounded) multivariable regression association (15,16).

**Association between adiponectin and metabolic outcomes.** To compare the result of instrumental variable analysis with a standard association test, we regressed each metabolic outcome against adiponectin levels using linear regression for continuous outcome variables and logistic regression for type 2 diabetes. We adjusted for age and sex in all studies and age, sex, and either BMI or triglyceride levels in a subset of studies (RISC, Genetics of Diabetes Audit and Research Tayside Scotland [GoDARTS], Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk [SAPHIR], FHS, and Cohorte Lausannoise [CoLaus]; $n$ = up to 11,829).

**Summary statistics genetic risk score.** We used a summary statistics genetic risk score calculated in each study using three available common SNPs associated with adiponectin levels (rs17300539, rs3774261, and rs3821799). We did not use rs17366653 because it was not well-imputed in these studies. We calculated the genetic risk score using summary statistics of phenotype–genotype association weighted by each SNP's corresponding effect size with adiponectin (17). We confirmed that this summary statistics genetic risk score was valid by calculating the score using individual level genotype data available in a subset of studies as below:

$$s_j = \sum_{i=1}^{4} w_i g_{ij},$$

where $s_j$ is the score for individual $j$, $g_{ij}$ is the number of risk alleles (0, 1, 2, or dosage of the risk allele) for SNP $i$ carried by individuals $j$, and $w_i$ is the effect size on adiponectin levels for SNP $i$ from the meta-analysis results of 13 studies (up to 33,671 individuals): $w_{rs17300539}$ (G as effect allele) = −0.330; $w_{rs3774261}$ (G as effect allele) = −0.354; and $w_{rs3821799}$ (T as effect allele) = −0.352. We performed a logistic regression with the outcome variable of type 2 diabetes status and exposure variable as genetic risk score and covariates including age, sex, and principle components or center or other measures required to correct for ethnicity.

**Summary statistics genetic risk score for fasting insulin-associated variants.** We used recently identified genetic variants associated with fasting insulin levels (18) to perform a reciprocal analysis to test the hypothesis that genetic determinants of insulin resistance (as measured by higher fasting insulin levels) are causally associated with lower circulating adiponectin levels. We used a summary statistics genetic risk score using 17 SNPs identified as associated with fasting insulin and or fasting insulin adjusted for BMI (18).

**Sensitivity analysis.** We performed two sets of sensitivity analyses: *1*) to assess whether or not associations differed between sexes, we repeated the inverse-variance meta-analyses in men and women separately (sex-difference *P* values were calculated by *t* tests); and *2*) since rs17366653 is predicted to alter the splicing pattern of adiponectin (13) and may produce different transcripts or proteins, we reran analyses excluding this SNP.

**Meta-analysis.** We performed meta-analysis using METAL 2009-10-10 release (19) and package metafor in R (20). Overall associations from observational analyses and instrumental variable analyses were evaluated across the studies with fixed-effects inverse variance–weighted meta-analysis. Heterogeneity statistics were calculated in the meta-analysis by the $I^2$ statistic, which is a measure of the variation in effect size attributable to heterogeneity (21). Random effects and meta-regression were used to allow for and explore associations with evidence of heterogeneity.

**Measures of insulin sensitivity.** For measures of insulin sensitivity, we used five studies (RISC, Eugene2, ULSAM, Stanford Insulin Suppression Test [IST], and Minnesota) and meta-analyzed results using the program METAL. In Eugene2, ULSAM, Minnesota, and RISC, insulin sensitivity was measured using the hyperinsulinemic-euglycemic clamp based protocol (22). In the Stanford study, insulin sensitivity was measured by the insulin suppression test with a readout of steady-state plasma glucose. The steady-state plasma glucose value is highly inversely correlated to M-value [$r$ = −0.87 (23) and −0.93 (24)], so meta-analysis was performed among the five studies by reversing the signs of the effect sizes in Stanford.

**Power calculation.** To assess the power of our study, we calculated the approximate number of individuals we would need to detect the expected instrumental variable (four *ADIPOQ* SNPs): fasting insulin or type 2 diabetes associations given the instrumental variable–adiponectin and adiponectin–fasting insulin or type 2 diabetes associations. We used the product of the

variance explained by the instrumental variable–adiponectin and adiponectin–fasting insulin or type 2 diabetes associations and a $P$ value of 0.01.

## RESULTS

**A combination of four *ADIPOQ* variants explained 4% of the variation in circulating adiponectin levels.** We identified four SNPs (rs17366653, rs17300539, rs3774261, and rs3821799) at the *ADIPOQ* locus that explained 4% variation in adiponectin levels in a multivariable analysis ($n$ = up to 33,671; Table 2 and Fig. 1). We did not observe any difference in these associations between males and females (Supplementary Figs. 2–5). These variants, used together as an instrument, provided us with >99% statistical power to detect associations that explain 0.1% variance at $P$ = 0.01. The figure of 0.1% variance is the product of the variance explained by the four SNPs (4%) and the variance explained between adiponectin and fasting insulin levels when corrected for BMI (correlation $r$ = 0.16; variance $r^2$ = 2.5%).

**Instrumental variables and summary statistics genetic risk score approaches provide no evidence of a causal association between circulating adiponectin and insulin resistance in up to 29,771 individuals.** Lower circulating adiponectin levels were strongly correlated with increased fasting insulin. A 1-SD decrease in adiponectin levels was associated with a 0.31 SD (95% CI 0.26–0.35) increase in fasting insulin ($P$ = 5E-40; Table 3 and Fig. 2*A*). In contrast, the instrumental variable analysis did not provide any evidence of a causal association between lower adiponectin and increased fasting insulin; the mean difference in fasting insulin per SD of adiponectin was 0.02 (95% CI −0.07 to 0.11; $P$ = 0.60; $n$ = 29,771) (Fig. 2*B*). The 95% CIs from the instrumental variable analysis clearly excluded the observational regression estimate (Fig. 3 and Table 3). The 95% CIs from the instrumental variables analysis also clearly excluded the observational regression estimate when adjusting for BMI (0.16 [95% CI 0.15–0.18]; $n$ = 11,829) or triglyceride levels (0.19 [0.17–0.20]; $n$ = 11,346). There was some evidence of heterogeneity (Table 3 and Supplementary Table 5) but meta-regression analysis, including the variables of average age, proportion of males, and average BMI, did not reduce heterogeneity (test of moderators, $P$ = 0.39). Sensitivity analyses did not appreciably change these estimates (Supplementary Table 6 and Supplementary Figs. 6 and 7).

Lower circulating adiponectin levels were strongly correlated with insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp in 2,109 individuals from the RISC, ULSAM, and Minnesota studies. A 1-SD decrease in adiponectin levels was associated with a 0.34-SD (95% CI 0.30–0.38; $P$ = 3E-61) decrease in M-value. We observed nominal evidence of a causal association between genetically lower adiponectin levels and insulin sensitivity (−0.20 SD [−0.38 to −0.02]; $P$ = 0.03) in 1,860 individuals from the ULSAM, RISC, and Minnesota studies in which adiponectin levels were measured and we could perform an instrumental variable analysis using three *ADIPOQ* SNPs. In contrast, a summary statistics genetic risk score (Supplementary Table 7) provided no evidence of a causal association between circulating adiponectin levels and insulin sensitivity in 2,969 individuals (−0.03 SD [−0.07 to −0.01]; $P$ = 0.12).

**A summary statistic genetic risk score approach provides evidence of a causal association between insulin resistance as measured by fasting insulin levels and lower circulating adiponectin levels.** We used 17 SNPs recently identified as associated with fasting insulin at the genome-wide significance level [by the Meta-Analyses of Glucose and Insulin Related Traits Consortium (18)] to test the reciprocal hypothesis that genetic determinants of insulin resistance (as measured by fasting insulin) causally influence circulating adiponectin. The fasting insulin summary statistics genetic risk score was strongly associated with adiponectin using >29,000 individuals (12) (per weighted fasting insulin raising allele was associated with a −0.01 SD ($P$ = 2E-20) change in adiponectin levels (Supplementary Fig. 8).

**A summary statistics genetic risk score approach provides no evidence of a causal association between circulating adiponectin and type 2 diabetes in 15,960 case subjects vs. 64,731 control subjects.** Lower adiponectin levels were strongly correlated with an increased risk of type 2 diabetes; a decrease of 1 SD in adiponectin levels was associated with an odds ratio of 1.75 (95% CI 1.47–2.13; $P$ = 5E-10) (Table 4 and Fig. 4*A*). Conversely, the analysis of the weighted adiponectin summary statistics genetic risk score, constructed based on three SNPs (rs17300539, rs3774261, and rs3821799), provided no evidence that individuals with lower genetically influenced adiponectin levels were at increased risk of type 2 diabetes (OR per weighted adiponectin lowering allele: 0.99 [0.95–1.04]; $P$ = 0.77; 15,960 case subjects vs. 64,731 control subjects). This result was consistent with an allele score calculated from a subset of five studies using individual-level genotype data (OR per weighted adiponectin-lowering allele: 1.03 [0.86–1.24]; 8,552 case subjects vs. 24,050 control subjects). We also observed no evidence of a causal association between genetically lower adiponectin levels and increased risk of type 2 diabetes (OR 0.94 [0.75–1.19]; $P$ = 0.61) in the 2,777 case subjects and 13,011 control subjects in whom we had adiponectin levels measured and could perform an instrumental variable analysis (Table 4 and Fig. 4*B*). The 95% CIs from the instrumental variable analysis clearly excluded the observational regression slope (Table 4). We observed heterogeneity in

TABLE 2
Associations between four SNPs and adiponectin levels in univariable and multivariable models from 13 European studies

| SNP | Alleles (effect/other) | Effect allele frequency | Univariable analysis | | | | Multivariable analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Effect (SD) | SE | $P$ value | $N$ | Effect (SD) | SE | $P$ value | $N$ |
| rs17300539 | A/G | 0.08 | 0.35 | 0.02 | 6E-115 | 35,031 | 0.32 | 0.02 | 2E-83 | 33,671 |
| rs17366653 | T/C | 0.98 | 0.59 | 0.03 | 6E-66 | 34,571 | 0.59 | 0.04 | 8E-62 | 33,599 |
| rs3774261 | A/G | 0.38 | 0.12 | 0.01 | 5E-49 | 34,662 | 0.35 | 0.02 | 3E-99 | 33,235 |
| rs3821799 | T/C | 0.43 | 0.02 | 0.01 | 0.005 | 34,700 | −0.34* | 0.02 | 5E-99 | 33,235 |

*The big change in the effect size is because the two SNPs are in partial linkage disequilibrium ($r^2$ = 0.7) and the adiponectin-decreasing alleles are on opposite haplotypes (i.e., rs3774261 and rs3821799 cancel each other out, as described previously for gene expression levels) (49).

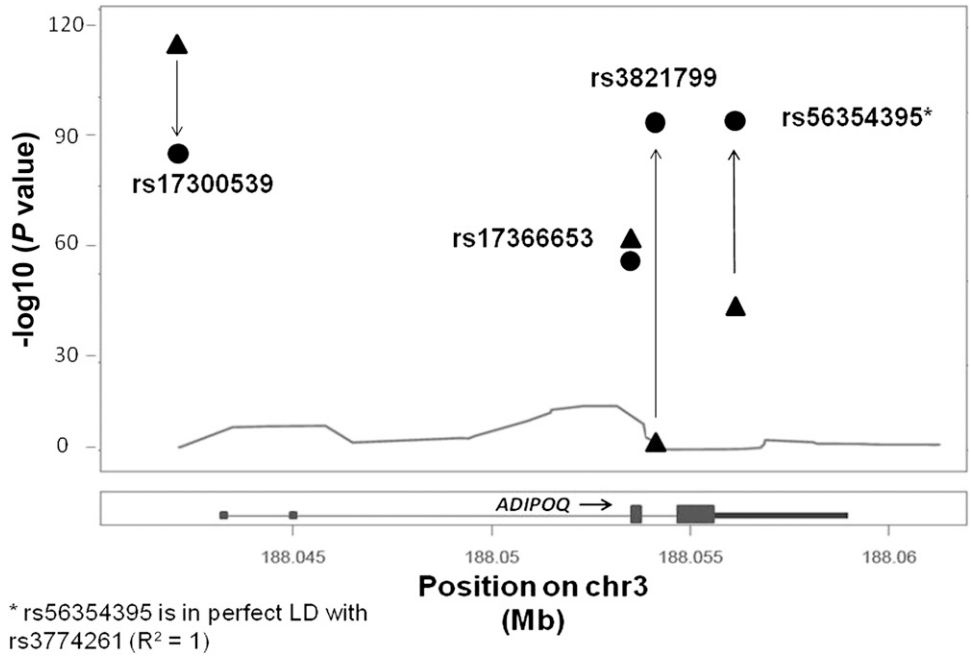


**FIG. 1. Adiponectin: SNP association in univariable analysis (triangles) and multivariable analysis (circles). chr3, chromosome 3; LD, linkage disequilibrium.**

observational analysis ($I^2$ = 90.4). Sensitivity analyses did not appreciably change these estimates (Supplementary Table 6 and Supplementary Figs. 9 and 10).

**An instrumental variables approach provides no evidence of a causal association between circulating adiponectin and other metabolic traits in up to 30,588 individuals.** Instrumental variable analyses did not provide any evidence that genetically decreased circulating adiponectin levels have a causal effect on fasting glucose, BMI, triglycerides, HDL-C, and cholesterol (Table 3). In all analyses, the 95% CIs from the instrumental variable analysis had no overlap with the 95% CIs from the observational analysis and clearly excluded the observational regression slope, except the analysis of LDL-C (observational 95% CI 0.03–0.10; instrumental variable 95% CI −0.03 to 0.09; Table 3). Sensitivity analyses did not appreciably change these estimates (Supplementary Table 6). We observed heterogeneity in observational analyses ($I^2$ 81.6–90.4), but meta-regression did not detect variables that reduced this heterogeneity.

**Non-European studies.** Using data from two Asian studies including the Cebu Longitudinal Health and Nutrition Study (CLHNS) and Cardiovascular Risk Factor Prevalence Study (CRISPS) (total $n$ = 2,991), we did not find any evidence of a causal effect of adiponectin on fasting insulin or risk of type 2 diabetes using one available SNP (rs6773957), which is in complete linkage disequilibrium with rs3774261 and rs3821799 in Asian populations. In the Jackson Heart Study (JHS) of African American individuals ($n$ = 2,053), none of the SNPs were associated with adiponectin levels.

## DISCUSSION

Our approach allowed us to plot a genetically determined regression line between adiponectin and secondary metabolic traits. Our study adds to the current literature, as it included a large enough number of individuals to confidently exclude the observational regression estimates for

TABLE 3
Associations between lower adiponectin levels and metabolic traits using linear regression and instrumental variable analysis (results from random effects meta-analysis)

| | Observational regression analysis | | | | | | | Instrumental variable analysis | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait | Effect (SD) | 95% LCI | 95% UCI | SE | *P* value | *N* | $I^2$ | Effect (SD) | 95% LCI | 95% UCI | SE | *P* value | *N* | $I^2$ |
| Fasting insulin | 0.31 | 0.26 | 0.35 | 0.02 | 5E-40 | 30,458 | 93.6 | 0.02 | −0.07 | 0.11 | 0.05 | 0.60 | 29,771 | 50.6 |
| BMI | 0.27 | 0.24 | 0.30 | 0.02 | 3E-59 | 31,277 | 87.5 | 0.02 | −0.07 | 0.10 | 0.04 | 0.70 | 30,588 | 48 |
| Fasting glucose | 0.14 | 0.11 | 0.17 | 0.01 | 1E-22 | 30,931 | 81.6 | 0.02 | −0.04 | 0.07 | 0.03 | 0.58 | 30,234 | 0 |
| Total cholesterol | −0.01 | −0.04 | 0.02 | 0.02 | 0.59 | 30,706 | 86.9 | 0.04 | −0.02 | 0.09 | 0.03 | 0.23 | 29,951 | 0 |
| HDL-C | −0.41 | −0.44 | −0.38 | 0.02 | 9E-158 | 30,651 | 86.1 | −0.06 | −0.12 | 0.06 | 0.03 | 0.06 | 29,899 | 11.6 |
| LDL-C | 0.06 | 0.03 | 0.10 | 0.02 | 9E-05 | 30,211 | 85.4 | 0.03 | −0.03 | 0.09 | 0.03 | 0.31 | 29,498 | 0 |
| Triglycerides | 0.28 | 0.25 | 0.32 | 0.02 | 2E-64 | 30,362 | 87.0 | 0.03 | −0.03 | 0.09 | 0.03 | 0.32 | 29,646 | 0.8 |

The effect value is the SD change in trait levels per 1-SD decreased adiponectin levels. LCI, lower CI; UCI, upper CI.

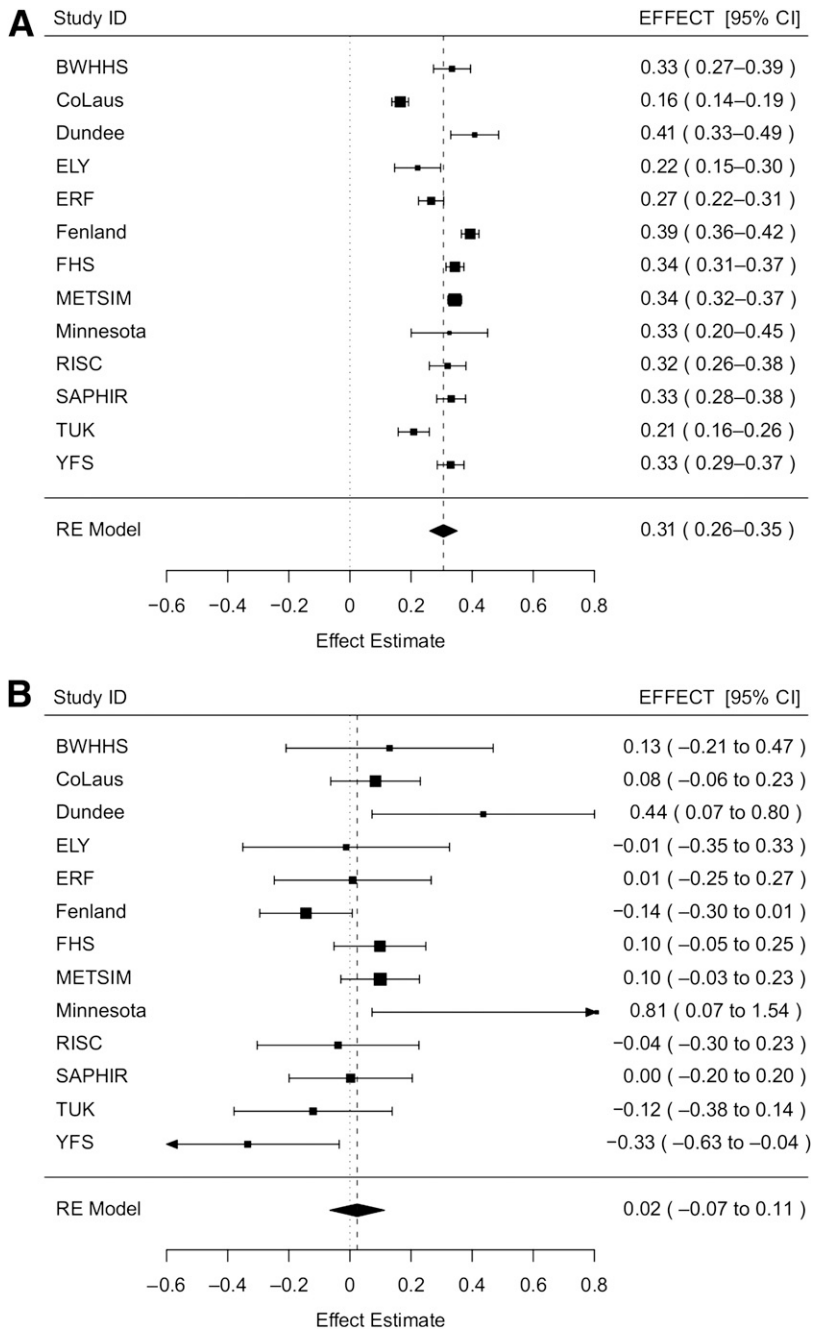


**FIG. 2. Forest plots of the associations between circulating adiponectin levels and fasting insulin in European studies. *A*: Meta-analysis of observational linear regression results of mean difference in fasting insulin per 1-SD lower adiponectin levels. *B*: Meta-analysis of instrumental variables results of mean difference in fasting insulin per 1-SD lower adiponectin levels. Although linear regression suggests a strong relationship between lower circulating adiponectin levels and increased fasting insulin, instrumental variable analysis does not support a causal association. In each plot, the dashed line indicates the effect size from the overall meta-analysis. The effects are for 1-SD decrease in adiponectin levels. RE, random effects.**

fasting insulin and type 2 diabetes. Limited sample size meant that we could not confidently include or exclude the observational regression estimates for insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp or insulin suppression tests. Previous studies studied fewer individuals, included variants likely to have pleiotropic effects, or did not conduct an instrumental variables analysis. Our results provided no evidence that genetically determined lower adiponectin levels increase insulin resistance, as assessed by fasting insulin, or type 2 diabetes risk. The 95% CIs around our instrumental variables estimate of the adiponectin–fasting insulin association excluded effects approximately one-third and above of the observed (age- and sex-adjusted) association between adiponectin and fasting insulin. Total circulating adiponectin levels are significantly higher in females than males (25,26), but our sex-dichotomized analyses did not show any evidence for differences between sexes in its association with fasting insulin, type 2 diabetes, or other outcomes.

A large number of studies have tested associations between *ADIPOQ* SNPs and insulin resistance and type 2 diabetes (13,27–38). Most of these studies have been

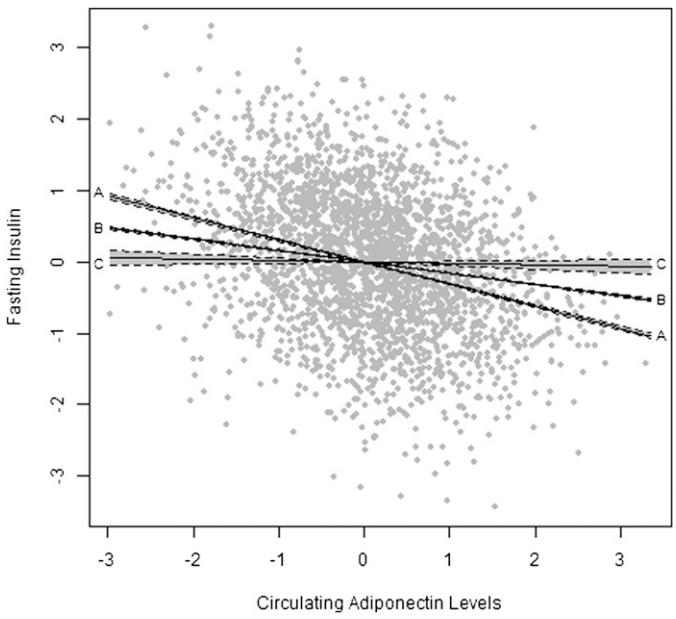


**FIG. 3. Comparison of linear relationships between circulating adiponectin levels and fasting insulin adjusted for age and sex (line A); age, sex, and BMI (line B); and when estimated using the four adiponectin SNPs together as an instrument (line C). The *x*- and *y*-axes represent circulating adiponectin levels and fasting insulin (both variables inverse-normal transformed), respectively. Light gray points represent a scatter plot of the correlation between circulating adiponectin levels and fasting insulin based on the data from three studies (RISC, GoDARTS, and BWHHS) in which individual level data were available. Gray areas constrained by dashed lines represent 95% CI around each estimate. Observational and instrumental variable slopes and CIs have been formulated based on the meta-analysis results of 13 studies.**

appreciably smaller than our study. The largest study (5,145 case subjects vs. 6,374 control subjects) that tested specifically the association between *ADIPOQ* SNPs and type 2 diabetes, and overlapped with our data, was negative (13). In a recent GWAS study of adiponectin levels, a multi-SNP allele risk score, calculated based on 196 SNPs from across the genome, was associated with type 2 diabetes risk and a number of related traits (12). Contrary to our results, these findings could be interpreted as providing causal evidence for the association of adiponectin with these outcomes. However, as the authors noted, their results may have been influenced by pleiotropy at loci other than *ADIPOQ* and therefore do not constitute a Mendelian randomization study. To clarify further the potentially confusing messages between our study and the adiponectin GWAS study, we tested the 10 SNPs associated with adiponectin levels outside of the *ADIPOQ* region and confirmed that they are associated with fasting insulin in the Meta-Analyses of Glucose and Insulin Related Traits Consortium study (18) The overall effect of non-*ADIPOQ* adiponectin-decreasing alleles was associated with a 0.24-SD increase in fasting insulin (95% CI 0.18–0.30; $P$ = 3E-14). This association, together with our null association of *ADIPOQ* SNPs, strongly suggests that the non-*ADIPOQ* SNPs operate through secondary or pleiotropic mechanisms. Our results add to a recent Mendelian randomization study that showed evidence of a causal association between adiponectin levels and insulin resistance assessed by euglycemic clamp in 942 men from ULSAM (10). Our meta-analysis of 1,860 individuals, including the ULSAM study, indicates that larger numbers will be needed to confidently include or exclude the observational association between adiponectin and insulin sensitivity. Testing insulin sensitivity in very large numbers, however, is not very feasible given the complexity and invasiveness of the physiological tests, and a combination of our summary statistics–based results in 2,969 individuals and the results with fasting insulin in 29,771 individuals suggest the weight of evidence is against a causal role of adiponectin in insulin resistance.

Although the conclusion that genetically determined low levels of adiponectin are not associated with increased risk of insulin resistance is at odds with the widely held view of adiponectin as an insulin-sensitizing hormone, the direct evidence supporting this notion comes largely from rodent models, and the situation in humans is more complex (39). Indeed, in humans with extreme insulin resistance due to loss of insulin receptor function, plasma adiponectin levels are often extremely high (40–44). Moreover in healthy volunteers, insulin infusion lowers plasma adiponectin (45), and in type 1 diabetes, it is elevated (46–48). Allied to other findings, including the observation that in a single family with insulin resistance, due to mutation of the intracellular signal transducer AKT2, adiponectin levels are very low (42), this has raised the possibility that the association between insulin resistance in humans may be explained by high levels of insulin suppressing adiponectin production through intact signaling pathways (39). In other words, it is possible to interpret current human data as providing evidence that it is the hyperinsulinemia caused by prevalent forms of insulin resistance that leads to low plasma adiponectin levels rather than vice versa. The current results, including the association between the fasting insulin raising genetic score and lower adiponectin levels, are consistent with this model.

Our study has limitations. First, the SNPs we used are associated with altered levels of adiponectin protein and not its function; we have tested the role of increased and decreased circulating adiponectin levels rather than its

TABLE 4
Associations between lower adiponectin levels and type 2 diabetes using logistic regression, instrumental variable analysis, allele score, and summary statistics genetic risk score

| Analysis | OR | 95% LCI | 95% UCI | *P* value | $I^2$ | *N* (case subjects vs. control subjects) |
|---|---|---|---|---|---|---|
| Logistic regression analysis | 1.75 | 1.47 | 2.13 | 5E-10 | 90.4 | 16,075 (2,851 vs. 13,224) |
| Instrumental variable analysis | 0.94 | 0.75 | 1.19 | 0.61 | 0 | 15,788 (2,777 vs. 13,011) |
| Summary statistics genetic risk score | 0.99 | 0.95 | 1.01 | 0.77 | 0 | 72,192 (15,960 vs. 64,731) |

Model includes rs17300539, rs3774261, and rs3821799. LCI, lower CI; UCI, upper CI.

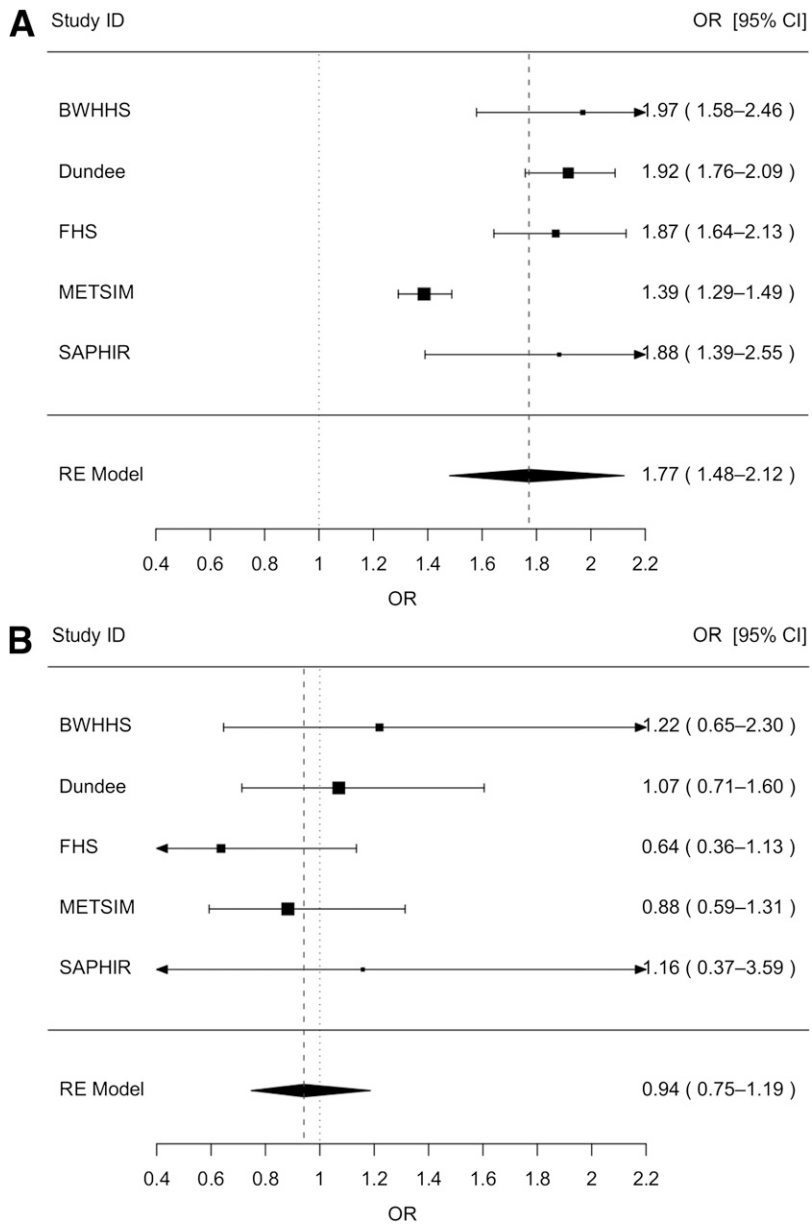


**FIG. 4. Forest plots of the associations between circulating adiponectin levels and type 2 diabetes risk in Europeans. *A*: Meta-analysis of observational linear regression results of OR of type 2 diabetes per 1-SD lower adiponectin levels. *B*: Meta-analysis of instrumental variables results of OR of type 2 diabetes per 1-SD lower adiponectin levels. Although linear regression suggests a strong relationship between lower circulating adiponectin levels and higher risk of type 2 diabetes, instrumental variable analysis does not support a causal association. In each plot, the dashed gray line indicates the effect size from the overall meta-analysis. The ORs are for 1-SD decrease in adiponectin levels. RE, random effects.**

function in other tissues such as the liver. Second, we cannot rule out a causal association between circulating adiponectin and insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp, and we cannot completely rule out a causal association between fasting-based measures of insulin resistance, because our study is consistent with a regression slope of 0.11 (the upper 95% CI of our instrumental variable estimate). Third, we observed appreciable heterogeneity between studies in our observational associations that mean our estimates of the nongenetic correlations are noisy. However, there was little heterogeneity in the genetic associations. Finally, the Mendelian randomization approach has limitations. For example, we cannot account for complex feedback loops or canalization, the body's adaptation to early physiological changes caused by subtle genetic changes. We cannot also rule out the possibility that the relationship between adiponectin and outcome metabolic traits varies by age or after diabetes diagnosis, potentially adding more noise to the instrumental variables analysis.

In summary, we have performed a Mendelian randomization study to test the causal role of lower adiponectin levels with increased insulin resistance and type 2 diabetes. Our results provide no consistent evidence that genetically influenced decreased circulating adiponectin levels increase the risk of insulin resistance or type 2 diabetes. These results do not provide any evidence that pharmaceutical and lifestyle interventions designed to alter adiponectin levels will improve insulin resistance or prevent type 2 diabetes.

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H.Y. designed the study, wrote the first draft of the manuscript, contributed to the writing and revision of the manuscript, performed the meta-analyses and other key analyses, and performed the statistical analyses for the British Women's Heart and Health Study (BWHHS), RISC, GoDARTS, and Wellcome Trust Case Control Consortium (WTCCC) studies. C.Lam. contributed to the writing and revision of the manuscript, performed the meta-analyses and other key analyses, and performed the statistical analyses for the SAPHIR study. R.A.S. contributed to the writing and revision of the manuscript and performed the statistical analyses of the Fenland and Ely studies. Z.D. contributed to the writing and revision of the manuscript, was involved in the design, and performed the statistical analyses of the TwinsUK (TUK) study. M.-F.H. contributed to the writing and revision of the manuscript and was involved in genotyping and performed the statistical analyses for the Framingham study. L.L.W. (CoLaus), A.S. (Metabolic Syndrome in Men [METSIM]), S.G.B. (JHS), P.H. (Erasmus Rucphen Family [ERF] study), Y.W. (CLHNS), C.Y.Y.C. (CRISPS), J.S.P. and N.Z. (Minnesota), J.S.P. (ARIC), A.U.J. and T.M.T. (Finland-United States Investigation of NIDDM Genetics [FUSION]), J.D., J.H., and C.-T.L. (Framingham), S.G. (ULSAM), and J.H.Z. (InterAct) performed the statistical analyses of the studies specified in parentheses. L.-P.L. performed the statistical analyses and was involved in genotyping of the Cardiovascular Risk in Young Finns (YF) study. P.H. was involved in sample collection, phenotyping, genotyping, and design of the ERF study. J.S.P. and A.R.S. (Minnesota), C.M.B. (ARIC), A.T.H. and M.I.M. (WTCCC), J.K. and M.L. (METSIM), and R.S.V. (Framingham) were involved in sample collection and phenotyping of the studies specified in parentheses. W.X. and E.F. (RISC), T.L.A., J.W.K., and T.Q. (Stanford), T.H., H.H., O.P., U.S., and M.L. (Eugene2), and K.H., C.M.L., J.P., and A.D.M. were involved in the GWAS of the euglycemic clamp. R.N.B., M.B., F.S.C., J.T., and K.L.M. (FUSION), F.e.B. (ERF), J.D. (Framingham), R.J.F.L. (Fenland and Ely), A.D.M. and C.N.A.P. (GoDARTS), A.R.S. (Minnesota), K.L.M. (CLHNS), A.B. (JHS), and D.M.W. (CoLaus) were involved in the design of the studies specified in parentheses. S.H.D. (JHS), C.G. (Fenland and Ely), A.D. (Framingham), and T.L. (YF) were involved in genotyping of the studies specified in parentheses. N.G.F. and N.J.W. were involved in sample collection, phenotyping, and design of the Fenland and Ely studies. M.K., T.L., J.S.V., and O.T.R. were involved in the sample collection, phenotyping, and design of the YF study. L.K. and F.K. were involved in sample collection, phenotyping, and genotyping of the SAPHIR study. J.J.N. and M.W. were involved in sample collection, phenotyping, and design of the RISC study and in GWAS of the euglycemic clamp. T.D.S. was involved in sample collection, phenotyping, and genotyping of the TUK study. K.W.v.D. performed the statistical analyses and was involved in genotyping of the ERF study. C.Lan. was involved in sample collection, phenotyping, and design of the Fenland and Ely studies. E.I. was involved in sample collection, phenotyping, genotyping, and design of the ULSAM study and in GWAS of the euglycemic clamp. R.K.S. contributed to the writing and revision of the manuscript. K.S.L.L. was involved in sample collection, phenotyping, and design of the CRISPS study. B.P. was involved in sample collection, phenotyping, genotyping, and design of the SAPHIR study. C.v.D. performed the statistical analyses and was involved in sample collection, phenotyping, and design of the ERF study. D.A.L. contributed to the writing and revision of the manuscript and was involved in the design of the BWHHS study. J.B.M. contributed to the writing and revision of the manuscript and was involved in sample collection, phenotyping and genotyping of the Framingham study. J.B.R. contributed to the writing and revision of the manuscript, and was involved in sample collection, phenotyping, and design of the TUK study. T.M.F. designed the study, contributed to the writing and revision of the manuscript, and was involved in genotyping of the GoDARTS study. T.M.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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