Mendelian Randomization Studies Do Not Support a Role for Raised Circulating Triglyceride Levels Influencing Type 2 Diabetes, Glucose Levels, or Insulin Resistance

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OBJECTIVE—The causal nature of associations between circulating triglycerides, insulin resistance, and type 2 diabetes is unclear. We aimed to use Mendelian randomization to test the hypothesis that raised circulating triglyceride levels causally influence the risk of type 2 diabetes and raise normal fasting glucose levels and hepatic insulin resistance.

RESEARCH DESIGN AND METHODS—We tested 10 common genetic variants robustly associated with circulating triglyceride levels against the type 2 diabetes status in 5,637 case and 6,860 control subjects and four continuous outcomes (reflecting glycemia and hepatic insulin resistance) in 8,271 nondiabetic individuals from four studies.

RESULTS—Individuals carrying greater numbers of triglyceride-raising alleles had increased circulating triglyceride levels (SD 0.59 [95% CI 0.52–0.65] difference between the 20% of individuals with the most alleles and the 20% with the fewest alleles). There was no evidence that the carriers of greater numbers of triglyceride-raising alleles were at increased risk of type 2 diabetes (per weighted allele odds ratio [OR] 0.99 [95% CI 0.97–1.01]; $P = 0.26$). In nondiabetic individuals, there was no evidence that carriers of greater numbers of triglyceride-raising alleles had increased fasting insulin levels (SD 0.00 per weighted allele [95% CI −0.01 to 0.02]; $P = 0.72$) or increased fasting glucose levels (0.00 [−0.01 to 0.01]; $P = 0.88$). Instrumental variable analyses confirmed that genetically raised circulating triglyceride levels were not associated with increased diabetes risk, fasting glucose, or fasting insulin and, for diabetes, showed a trend toward a protective association (OR per 1-SD increase in $\log_{10}$ triglycerides: 0.61 [95% CI 0.45–0.83]; $P = 0.002$).

CONCLUSIONS—Genetically raised circulating triglyceride levels do not increase the risk of type 2 diabetes or raise fasting glucose or fasting insulin levels in nondiabetic individuals. One explanation for our results is that raised circulating triglycerides are predominantly secondary to the diabetes disease process rather than causal. ***Diabetes*** **60:1008–1018, 2011**

Raised circulating triglyceride levels are strongly correlated with insulin resistance, raised glucose levels, and type 2 diabetes (1–8), but the causal nature of these associations is unclear because of the complex interactions between fat, muscle, and liver insulin resistance, dyslipidemia, and insulin secretion by β-cells.

Several lines of evidence suggest that raised triglyceride levels could causally influence the risk of type 2 diabetes, high glucose levels, and insulin resistance. Accumulation of triglycerides in tissues other than adipose has been proposed to result in lipotoxicity, a process that may increase the risk of type 2 diabetes. For example, excess triglycerides in the liver causes fatty liver disease and is thought to impair hepatic insulin signaling, resulting in insulin resistance (reviewed in [9]), whereas exposure of the β-cell to free fatty acids (FFAs) is thought to impair insulin secretion (10–13).

Epidemiological data support a possible etiological role for raised triglyceride levels in insulin resistance and type 2 diabetes. Raised serum triglycerides predict incident type 2 diabetes independently of BMI (1–4,6,14–16), although prospective evidence does not rule out the possibility that early disease processes can influence such associations. Data from some trials show that individuals receiving lipid-lowering therapies are less likely to develop type 2 diabetes (14,17–19). These findings have led to the proposal that therapies that lower circulating triglycerides could be used to improve insulin sensitivity and reduce the risk of type 2 diabetes (20–22).

One useful method to help dissect the causal nature of the correlations between metabolic traits is Mendelian randomization (23). This approach uses the principle that the random assortment of genotypes in meiosis is independent of nongenetic factors, including environmental risk factors, confounding factors, or disease processes. There are good proof-of-principle examples of Mendelian

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randomization. These include the association between *FTO* genotypes, which are robustly associated with total fat mass, and type 2 diabetes and blood pressure, which confirmed the causal associations between adiposity and these outcomes (24,25), and the association between LDL cholesterol–associated variants and heart disease (26).

In this study, we extend the Mendelian randomization approach to test the hypothesis that raised circulating triglyceride levels have an etiological role in type 2 diabetes, raised fasting glucose levels, and fasting-based measures of insulin resistance.

RESEARCH DESIGN AND METHODS

Type 2 diabetes case-control study. We studied 12,497 individuals (5,637 type 2 diabetic patients and 6,860 control subjects) from the Genetics of Diabetes Audit and Research in Tayside Scotland (Go-DARTS) study (27), a cross-sectional study that includes measures of circulating lipids, often with repeated measurements in the same individual (Table 1). Patients were excluded if their age at diagnosis was <35 or >70 years or if they needed insulin treatment within 1 year of diagnosis. For 2.1% of patients, age at diagnosis was not known, in which case those aged <45 years at the time of study were excluded. Control status was defined if individuals were between 35 and 80 years of age with an A1C <6.4% and/or fasting glucose <7 mmol/L. Analyses of associations involving triglyceride levels were limited to the 9,693 individuals (3,976 patients and 5,717 control subjects) that had triglyceride levels measured prior to taking any lipid-lowering medication. Of these individuals, 46.88% (74.72% of patients and 27.51% of control subjects) had more than one measure of triglycerides, in which case we used mean values.

Fasting-based measures of insulin resistance and glucose levels. For the study of continuous traits, we examined nondiabetic individuals from four studies. These studies were the Exeter Family Study of Childhood Health (EFSOCH) (28), the Go-DARTS study, the Fenland Study (29 Supplementary Information), and the British Women's Heart and Health Study (BWHHS) (30) (Table 1). The EFSOCH study consisted of parents of babies born between 2000 and 2004 from Exeter, U.K. For EFSOCH mothers, we used fasting measures taken postpregnancy. The Go-DARTS subjects are a subset of those studied as control subjects in the type 2 diabetes study described above, who had fasting glucose, insulin, and nonfasting lipid measures available (fasting lipid measures were not taken). The Fenland Study is a population-based study in the East Cambridgeshire and Fenland areas of the U.K. The BWHHS is a prospective cohort study of women aged 60–79 years recruited from 23 towns across Britain from 1999 to 2000.

We only included individuals with fasting glucose values <7.0 mmol/L. None of the individuals in the EFSOCH study, 26 (<2%) in the Fenland Study, and 5% in the BWHHS were on lipid-lowering medications. We did not use triglyceride measures from individuals on lipid-lowering medications in the Go-DARTS study. Details of fasting glucose and fasting insulin measurement methods are given in Supplementary Table 1. We calculated additional fasting-based measures of insulin resistance and β-cell function using the homeostasis model assessment of β-cell function (HOMA-B) and HOMA of insulin resistance (HOMA-IR) using the HOMA calculator (available at http://www.dtu.ox.ac.uk).

Selection of single nucleotide polymorphisms, genotyping, and quality control. We initially selected 12 independent single nucleotide polymorphisms (SNPs) that are associated with circulating triglyceride levels at genome-wide levels of significance ($P < 5 \times 10^{-8}$) (31–35). We excluded two of these SNPs from our analyses (*FADS1*-rs174547 and *GCKR*-rs1260326) because they are strongly associated with several other quantitative traits relevant to diabetes (29,36,37).

We genotyped 10 selected SNPs in the four studies using either a modified Taqman assay, a KASPAR assay (http://www.kbioscience.co.uk), directly or imputed genotypes from the Affymetrix GeneChip Human Mapping 500 K array, or the Illumina Human CVD array (Supplementary Methods). The genotyping success rate for each SNP was >92% in all studies, and the concordance rate between duplicates (at least 7% of samples) was at least 97%. All 10 variants were in Hardy-Weinberg equilibrium in each of the four studies ($P > 0.05$).

Statistical analyses. We used two approaches to assess the relationship between circulating triglyceride levels and diabetes-related outcomes: the triangulation approach outlined in Fig. 1 and an instrumental variable approach (38). All statistical analyses were performed using Stata/SE version 10.1 for Windows (StataCorp, Brownsville, TX). Meta-analyses were performed using the inverse-variance weighted fixed-effects estimator implemented in the Stata command, "metan."

TABLE 1
Clinical characteristics of individuals in four studies of continuous traits and case and control subjects of the Go-DARTS type 2 diabetes study

	Continuous-traits studies				Type 2 diabetes case-control studies	
	BWHHS	EFSOCH	Fenland	Go-DARTS*	Go-DARTS (control subjects)	Go-DARTS (type 2 diabetic case subjects)
n†	2,971	1,295	1,362	2,643	6,860	5,637
Age‡	68.8 ± 5.5	33.9 ± 6.0	44.9 ± 7.2	57.9 ± 11.4	59.3 ± 11.2	63.7 ± 9.3
BMI (kg/m^2)	27.24 ± 4.72	25.52 (22.99–28.33)	27.01 (4.81)	26.60 (23.90–29.50)	27.31 (24.71–30.51)	30.76 (27.54–34.71)
Male/female (%)	0/100	64/36	44/56	47/53	49.7/50.3	57.8/42.2
Triglycerides (mmol/L)	1.57 (1.20–2.15)	1.03 (0.75–1.54)	1.00 (0.70–1.50)	1.18 (0.86–1.71)	1.33 (0.94–1.93)	2.28 (1.63–3.22)
LDL cholesterol (mmol/L)	4.10 (3.44–4.84)	2.89 ± 0.84	3.39 ± 0.87	3.28 ± 0.88	3.22 ± 0.91	3.14 ± 0.95
HDL cholesterol (mmol/L)	1.60 (1.35–2.00)	1.47 ± 0.38	1.47 ± 0.40	1.60 ± 0 0.42	1.53 (1.26–1.85)	1.16 (0.98–1.36)
Total cholesterol (mmol/L)	6.60 (5.90–7.40)	4.97 ± 0 0.93	5.37 ± 1.00	5.53 ± 0 0.92	5.62 ± 0.99	5.76 ± 1.18
Fasting plasma insulin (pmol/L)	46.06 (30.56–63.89)	44.2 (32.7–66.92)	38.25 (25.80–57.10)	48.61 (34.72–69.45)	NA	NA
Fasting plasma glucose (mmol/L)	5.71 ± 0.48	4.67 ± 0.43	4.85 ± 0.48	4.86 ± 0.47	NA	NA
HOMA-B	63.9 (49.80–82.80)	107.60 (84.80–140.50)	80.75 (65.00–104.00)	100.50 (78.70–130.90)	NA	NA
HOMA-IR	0.83 (0.59–1.23)	0.93 (0.68–1.40)	0.71 (0.48–1.07)	1.01 (0.64–1.51)	NA	NA

Data are means ± SD for normally distributed variables and median (interquartile range) for skewed variables. *Note that Go-DARTS individuals used in the continuous-traits analyses are a subset of the control subjects used in the type 2 diabetes case-control analyses. †Number who met the inclusion criteria in addition to triglycerides, age, sex, and genotype for at least one SNP available except for the type 2 diabetes case-control study, in which individuals without triglyceride measures were also included. ‡Age at recruitment reported. NA, not applicable.

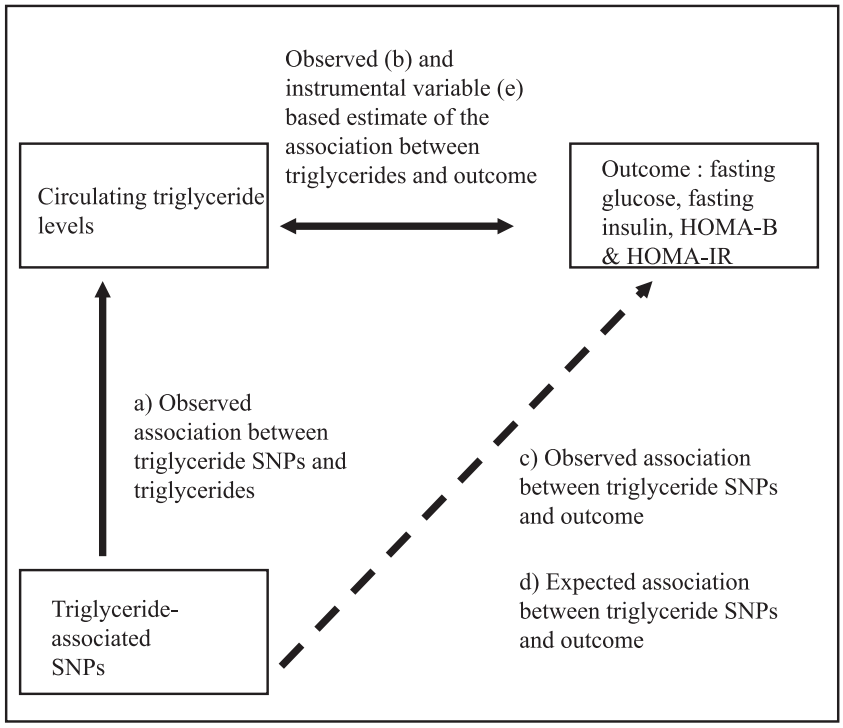

FIG. 1. Triangulation approach used to estimate the expected association for the SNP vs. type 2 diabetes or continuous trait (*d*) given the SNP versus triglyceride association (*a*) and the triglyceride versus type 2 diabetes or continuous trait associations (*b*).

Observed association between triglyceride SNPs and triglyceride levels. In each study, triglyceride levels (mmol/L) were $\log_{10}$ transformed before analysis. For the type 2 diabetes study, we generated age- and sex-corrected *z* scores of $\log_{10}$-transformed triglycerides, using all case and control subjects. To estimate the SNP versus triglyceride associations, we assumed a prevalence rate of 5% for type 2 diabetes in the U.K., and to be more representative of this general population we gave a weight of 95% to control subjects and 5% to case subjects. For continuous traits, we generated within-study *z* scores of $\log_{10}$-transformed triglycerides using the means and SD of the samples, where age, sex, triglyceride levels, and genotypes from at least eight of 10 SNPs were available.

Using both individual SNPs and a weighted allele score, we tested associations between genotypes and triglyceride levels. To create the weighted allele score, we used individuals with genotypes available from at least eight of 10 SNPs and accounted for the varying effect sizes of each SNP using equation 1, where w is the β-coefficient from the individual regressions of the SNP genotype against triglycerides.

$$\text{Weighted score} = w_1 \times \text{SNP}_1 + w_2 \times \text{SNP}_2 + \ldots\ldots\ldots\ldots + w_n \times \text{SNP}_n \quad (1)$$

We rescaled the weighted score to reflect the number of available SNPs (ranging from 8 to 10) using equation 2, as described in Lin et al. (39). For all further tests, we used this allele score.

$$\text{Allele score} = \frac{\text{Weighted score} \times \text{Number of SNPs available}}{\text{Sum weights of the available SNPs}} \quad (2)$$

We used this allele score as the independent variable and the $\log_{10}$-triglyceride *z* score as the dependent variable, and for the study of continuous traits we also used age and sex as covariates in linear regression analyses. In addition, we stratified individuals in each study into quintiles consisting of the 20% of individuals with increasing numbers of (weighted) triglyceride-raising alleles.

Observed association between triglycerides and outcomes. Using 3,976 case and 5,717 control subjects from the Go-DARTS study, we estimated the odds ratio (OR) for type 2 diabetes per 1-SD increase in $\log_{10}$-triglyceride *z* score in a logistic regression analysis. For the four nondiabetes studies, we tested four continuous-outcome variables: fasting glucose, fasting insulin, HOMA-B, and HOMA-IR. We $\log_{10}$ transformed the outcome variables that were skewed and created *z* score within each study. We used the $\log_{10}$-triglyceride *z* score as the independent variable and each outcome *z* score as the dependent variable, with age and sex as covariates in linear regression analyses prior to meta-analysis.

Observed association between triglyceride SNPs and outcomes. To test the association between triglyceride SNPs and type 2 diabetes, we used individual SNPs or the allele score as the independent variable and type 2 diabetes status as the dependent variable in logistic regression analyses, with age and sex as covariates. To test the association between triglyceride SNPs and continuous outcomes, we performed the same analyses but in linear regression models prior to meta-analysis.

Calculation of the approximate expected effect size of the association between triglyceride SNPs and outcomes. If raised triglyceride levels are etiologically associated with the outcomes, then under certain assumptions we would expect the point estimate of the expected outcome (a per-allele OR for type 2 diabetes, or SD effect size for continuous traits; Fig. 1*d*) to be a function of *1*) the SNP-triglyceride association and *2*) the triglyceride-outcome association (i.e., *d* = SD effect size of *a* × SD effect size/OR of *b* in Fig. 1). SEs for the expected effect sizes were calculated using the Taylor series expansion of the ratio of two means (40).

Instrumental variable analysis. To estimate the causal effect of triglycerides on outcomes, we performed instrumental variable analyses (Supplementary Methods and Supplementary Fig. 2). An instrumental variable analysis relates the variation in the potentially causal risk factor of interest (here, circulating triglyceride levels) that is influenced by the "instrument" (here, triglyceride genotypes) to the outcome (here, type 2 diabetes, fasting insulin, or fasting glucose levels). This method makes the assumption that the instrumental variable is not associated with measured or unmeasured confounders (likely to be true for genetic variants [38]) and is only related to the outcome via its effect on the risk factor. This produces an estimate of the causal effect in a similar way as an intention-to-treat analysis in a randomized controlled trial (38).

Instrumental variable analysis for type 2 diabetes case-control status. We limited this analysis to the 8,335 individuals (3,090 case and 5,245 control subjects) who had triglyceride levels measured prior to taking any lipid-lowering medication and genotypes from at least eight of 10 triglyceride SNPs. Instrumental variable analysis was performed using a logistic control function estimator (41). The analysis was performed in two stages. In the first stage, we assessed the observational association between allele score and triglyceride *z* score, as described in Fig. 1*a*. We saved the predicted values and residuals from this regression model. In the second stage, we used the predicted values from stage 1 as the independent variable (reflecting an unconfounded estimate of triglyceride levels attributed to these genotypes) and

diabetes status as the dependent variable in a logistic regression analysis. The residuals from stage 1 were included as a covariate, representing residual variation in triglyceride levels that is not attributed to these genotypes (41). We then used a Wald test to assess the evidence of a difference between the predicted-values coefficient (instrumental variable estimate of the causal effect of triglyceride levels on type 2 diabetes) and the residuals coefficient as test of endogeneity.

Instrumental variable analyses for fasting insulin, fasting glucose, HOMA-B, and HOMA-IR. We performed the instrumental variable estimation for each outcome in each study using the two-stage least-squares estimator, implemented in the Stata command "ivreg2." We tested for a difference between the instrumental variable and observational estimates using the Durbin-Wu-Hausman test of endogeneity. We meta-analyzed the instrumental variable estimates for each outcome from the individual studies.

Effects of triglyceride SNPs on HDL, LDL, and total cholesterol and effects when including *GCKR* and *FADS1* SNPs. We performed additional analyses, including tests on other lipid parameters to assess whether the results are predominantly driven by the variants' effects on triglycerides. This was assessed by tests, including only the four SNPs with the weakest effects on HDL cholesterol relative to their effects on triglycerides (the SNPs in or near *MLXIPL*, *ANGPTL3*, *NCAN*, and *TRIB1*) in the allele score. We also assessed the effects when including the *GCKR* and *FADS1* SNPs in the allele score and the effects when adjusting for BMI in addition to age and sex (Supplementary Methods).

RESULTS

Observed association between triglyceride SNPs and triglycerides. Associations between individual SNPs and triglycerides, meta-analyzed across each of the four studies with nondiabetic individuals, and separately for the type 2 diabetes study, are shown in Table 2. The majority was highly significantly associated with circulating triglyceride levels, and all effects were consistent with those reported in genome-wide association studies. Individuals carrying greater numbers of (weighted) triglyceride-raising alleles had increased circulating triglyceride levels (Table 2, Fig. 2*A*, and Supplementary Fig. 1). For example, the group of individuals in the highest quintile of the weighted allele score had triglyceride levels that were 0.59 SDs (95% CI 0.52–0.65) higher than those in the lowest quintile. There was some evidence (I^2 = 78.6%, P = 0.003) for heterogeneity between studies for the allele score–triglyceride association (Supplementary Fig. 1), but a random-effects meta-analysis resulted in a similar point estimate (data not shown).

Observed association between triglycerides and outcomes. A 1-SD increase in $\log_{10}$-triglyceride levels was associated with an OR of 2.68 (95% CI 2.54–2.82) for type 2 diabetes in the Go-DARTS study. Triglyceride levels were associated with each of the four continuous outcomes across the four nondiabetes studies. A 1-SD increase in triglyceride levels was associated with 0.12 SDs (95% CI 0.1–0.15), 0.36 SDs (0.33–0.38), 0.41 SDs (0.38–0.43), and 0.40 SDs (0.38–0.42) higher fasting glucose, HOMA-B, fasting insulin, and HOMA-IR, respectively (Table 3 and Fig. 3*A*). There was some evidence for heterogeneity between the nondiabetic studies for the associations involving fasting insulin (I^2 = 74.6%, P = 0.008), fasting glucose (I^2 = 69.5%, P = 0.02), and HOMA-IR (I^2 = 75.7%, P = 0.006). Random-effects meta-analyses resulted in similar point estimates (data not shown).

Observed association between triglyceride SNPs and type 2 diabetes. The details of the individual associations between triglyceride SNPs and type 2 diabetes are given in Table 4. None of the SNPs were associated with type 2 diabetes ($P > 0.01$). There was no evidence that individuals

TABLE 2
The association of individual SNPs and combinations of SNPs with circulating triglyceride levels from a meta-analysis of four studies of nondiabetic individuals and the Go-DARTS type 2 diabetes case-control study

		SNP(s) vs. triglycerides (continuous-traits meta-analysis)†			SNP(s) vs. triglycerides (type 2 diabetes case-control analysis)	
SNP/weighted allele score	Nearest gene*	Triglyceride z score per allele (95% CI)	P	Heterogeneity P (I^2%)	Triglyceride z score per allele (95% CI)	P
rs2954029	*TRIB1*	0.10 (0.07–0.13)‡	6×10^{-12}	0.19 (37.5)	0.08 (0.05–0.12)‡	4×10^{-7}
rs714052	*MLXIPL*	0.15 (0.11–0.20)‡	2×10^{-11}	0.81 (0.0)	0.13 (0.08–0.18)‡	1×10^{-7}
rs7557067	*APOB*	0.05 (0.02–0.09)‡	0.002	0.62 (0.0)	0.07 (0.03–0.10)‡	0.001
rs17216525	*NCAN*, *CLIP2*, *PBX4*	0.11 (0.05–0.16)‡	8×10^{-5}	0.42 (0.0)	0.04 (−0.02 to 0.10)‡	0.16
rs10889353	*ANGPTL3*	0.06 (0.03–0.09)‡	2×10^{-4}	0.36 (6.8)	0.05 (0.01–0.08)‡	0.008
rs7679	*PLTP*	0.05 (0.02–0.09)‡	0.005	0.41 (0.0)	0.06 (0.01–0.10)‡	0.008
rs7819412	*XKR6-AMAC1L2*	0.03 (0.00–0.06)‡	0.043	0.82 (0.0)	0.01 (−0.02 to 0.04)‡	0.54
rs328	*LPL*	0.21 (0.16–0.26)‡	4×10^{-17}	0.38 (2.1)	0.21 (0.16–0.27)‡	2×10^{-15}
rs3135506	*APOA5*	0.24 (0.18–0.30)‡	1×10^{-14}	0.33 (13.0)	0.17 (0.10–0.25)‡	5×10^{-6}
rs662799	*APOA5*	0.25 (0.18–0.31)‡	1×10^{-14}	0.60 (0.0)	0.17 (0.10–0.27)‡	1×10^{-6}
Allele score		0.12 (0.10–0.13)§	9×10^{-76}	0.003 (78.6)	0.09 (0.08–0.11)§	2×10^{-41}
Q2 vs. Q1		0.22 (0.15–0.28)‖	2×10^{-11}	0.93 (0.0)	0.17 (0.10–0.24)‖	4×10^{-6}
Q3 vs. Q1		0.32 (0.26–0.39)‖	3×10^{-23}	0.78 (0.0)	0.29 (0.21–0.36)‖	5×10^{-15}
Q4 vs. Q1		0.38 (0.32–0.45)‖	3×10^{-32}	0.85 (0.0)	0.33 (0.26–0.40)‖	5×10^{-19}
Q5 vs. Q1		0.59 (0.52–0.65)‖	2×10^{-72}	0.16 (41.3)	0.43 (0.36–0.50)‖	4×10^{-31}

Q = quintile of weighted allele score. The sample size in the allele score vs. triglyceride association was 8,084 and 8,335 in meta-analyses of the four continuous-outcome studies and Go-DARTS type 2 diabetic case and control subjects, respectively. For quintiles of allele score versus triglyceride analyses, the sample sizes ranged from 3,222 to 3,240 in continuous-traits meta-analyses and 3,315 to 3,372 in Go-DARTS type 2 diabetic case and control subjects. *Nearest gene information reported as in Kathiresan et al. (31), except for rs328, which is from Kathiresan et al. (34) and for rs3135506 and rs662799, which are from Pennacchio et al. (32). †Results from the continuous-traits meta-analysis and type 2 diabetes case-control analysis are not independent. A subset of control subjects from the type 2 diabetes case-control study are used in the continuous-traits study (those with fasting glucose <7.0 mmol/L and fasting insulin, triglycerides, and 8 of the 10 SNPs available). The effect sizes reported are ‡change in triglyceride z score per triglyceride-raising allele for individual SNPs, §change in triglyceride z score per unit increase in weighted allele score, or ‖difference in triglyceride z score between the relevant quintiles of the weighted allele score.

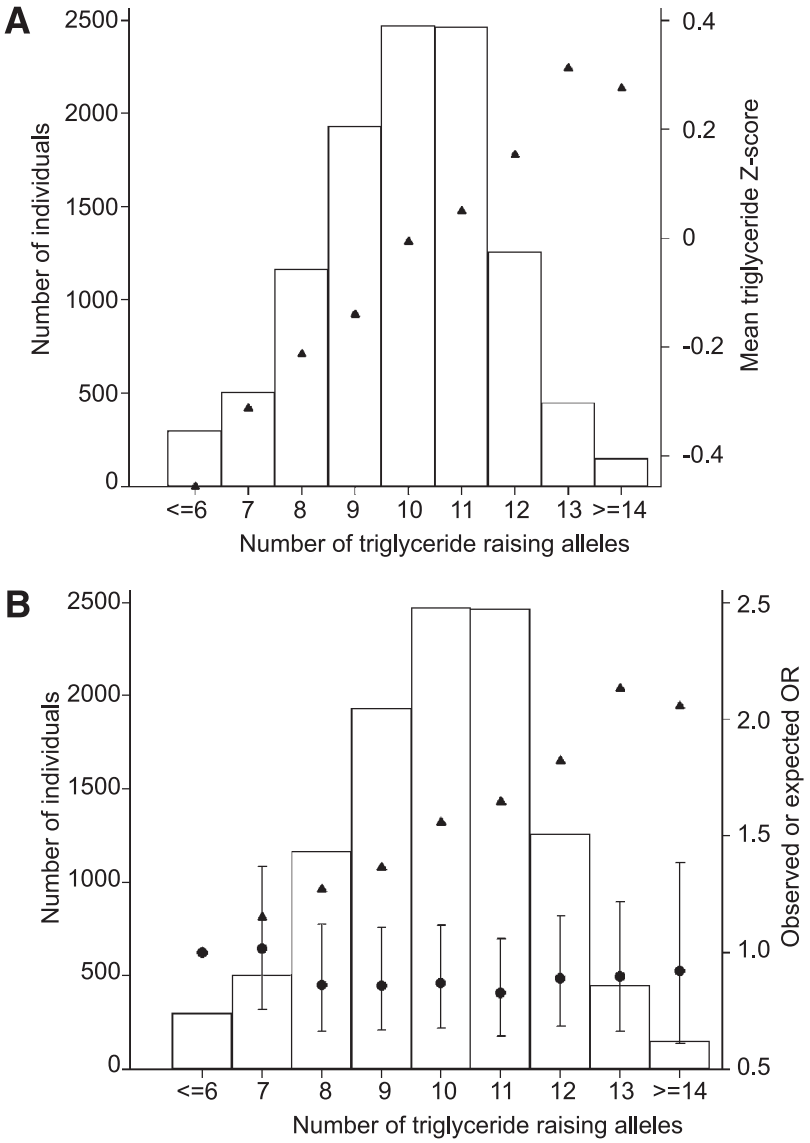

FIG. 2. The combined impact of the 10 triglyceride-associated SNPs on circulating triglyceride levels in the Go-DARTS study type 2 diabetic case and control subjects, with triangles representing the mean triglyceride *z* score within each triglyceride-increasing allele group (*A*); and observed (circles) and expected (triangles) type 2 diabetes ORs when comparing each allele group to a reference group of those with six or fewer triglyceride-raising alleles, with 95% CIs given for the observed data points (*B*). Participants were grouped by total number of triglyceride-raising alleles at all 10 SNPs, and the bars represent the number of individuals in each group.

carrying greater numbers of (weighted) triglyceride-raising alleles were at increased risk for type 2 diabetes (Table 4 and Fig. 2*B*).

Observed association between triglyceride SNPs and fasting insulin, fasting glucose, HOMA-B, and HOMA-IR. Associations between individual SNPs and each continuous outcome, meta-analyzed across the four non-diabetic studies, are given in Table 5 and Supplementary Table 2. None of the SNPs were associated with any of the four outcomes except for rs7819412 (*XKR6-AMAC1L2* locus), where there was some evidence for a positive association with fasting insulin (P = 0.004) and HOMA-IR (P = 0.004). There was no evidence that carriers of greater numbers of (weighted) triglyceride-raising alleles were at risk for increased fasting glucose or fasting insulin levels (Table 5, Fig. 3*B*, and Supplementary Table 2). There was no heterogeneity between studies except for the allele score–glucose association (I^2 = 80.9%, P = 0.001) and removing the one study influencing this heterogeneity, Go-DARTS, resulted in a nominal association between allele score and raised fasting glucose (P = 0.03).

Expected effect size of the association between triglyceride SNPs and type 2 diabetes. Estimates of the expected ORs and 95% CIs for the allele score–type 2 diabetes association are shown in Table 4**.** For the allele score and each quintile comparison, the 95% CIs of the observed ORs excluded the expected point estimate ORs estimated from the function of the SNP–triglyceride and triglyceride–type 2 diabetes correlations and vice versa.

Expected effect size of the association between triglyceride SNPs and fasting insulin, fasting glucose, HOMA-B, and HOMA-IR. Estimates of the expected effect sizes and the 95% CIs for the allele score–continuous outcome associations are given in Table 5 and Supplementary Table 2. For the allele score and each quintile comparison,

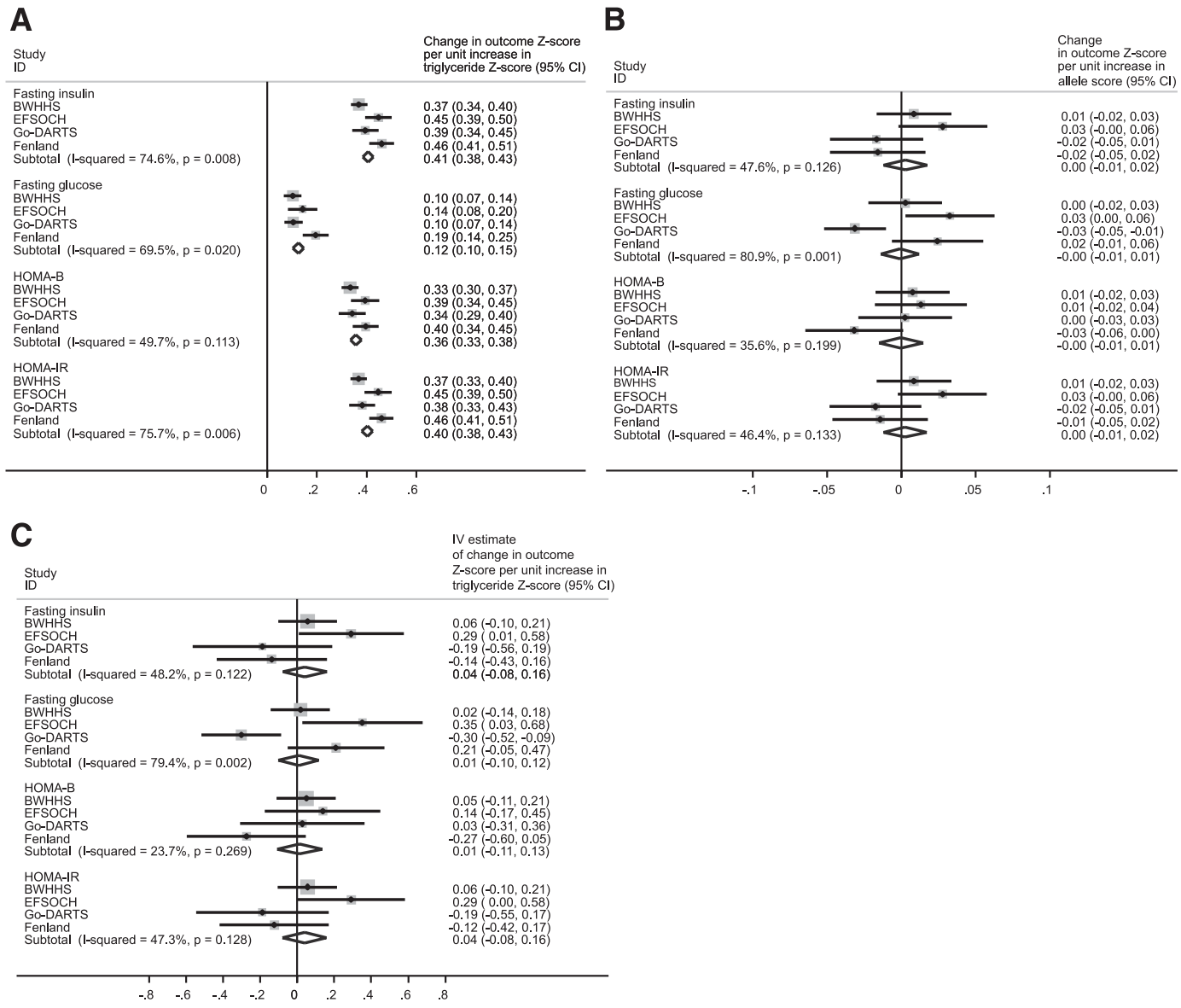

FIG. 3. Meta-analysis of continuous traits. Triglyceride–outcome associations (*A*), weighted triglyceride allele score–outcome associations (*B*), and instrumental variable analyses of triglyceride–outcome associations (*C*), all corrected for age and sex across the four studies of nondiabetic individuals.

the 95% CIs of the observed effect sizes excluded the approximate expected effect sizes estimated from the function of the SNP–triglyceride and triglyceride–outcome correlations and vice versa.

Instrumental variable estimate for type 2 diabetes. Instrumental variable estimation provided strong evidence that raised circulating triglyceride levels do not causally result in an increased risk of type 2 diabetes. Instead, there

TABLE 3
Meta-analysis results of observed and instrumental variable analyses of triglyceride–continuous outcome associations

	Observed change in outcome *z* score per 1-SD increase in $\log_{10}$-triglycerides			Instrumental variable estimate of change in outcome *z* score per 1-SD increase in $\log_{10}$-triglycerides		
Trait	Effect size (95% CI)	*P*	Heterogeneity *P* (I^2%)	Effect size (95% CI)	*P*	Heterogeneity *P* (I^2%)
Fasting insulin	0.41 (0.38–0.43)	<0.001	0.008 (74.6)	0.04 (−0.08 to 0.16)	0.49	0.12 (48.2)
Fasting glucose	0.12 (0.10–0.15)	<0.001	0.02 (69.5)	0.01 (−0.10 to 0.12)	0.90	0.002 (79.4)
HOMA-IR	0.40 (0.38–0.42)	<0.001	0.006 (75.7)	0.04 (−0.08 to 0.16)	0.51	0.13 (47.3)
HOMA-B	0.36 (0.33–0.38)	<0.001	0.11 (49.7)	0.01 (−0.11 to 0.13)	0.83	0.27 (23.7)

The sample sizes for the triglyceride vs. outcome associations ranged from 6,705 to 8,227 and from 6,519 to 8,040 for instrumental variable meta-analyses.

TABLE 4
The association of individual and combinations of SNPs with type 2 diabetes in the Go-DARTS type 2 diabetes case-control study

SNP	Nearest genes*	Type 2 diabetes OR per allele (95% CI)	*P*	Expected OR† = $a \times b$ (95% CI)
rs108893353	*ANGPTL3*	0.97 (0.91–1.03)	0.27	
rs17216525	*NCAN, CLIP2, PBX4*	0.89 (0.80–0.99)	0.03	
rs2954029	*TR1B1*	1.01 (0.95–1.06)	0.83	
rs714052	*MLXIPL*	0.96 (0.89–1.05)	0.38	
rs7557067	*APOB*	1.00 (0.93–1.07)	0.99	
rs7679	*PLTP*	0.98 (0.91–1.06)	0.64	
rs7819412	*XKR6-AMAC1L2*	0.97 (0.91–1.03)	0.26	
rs328	*LPL*	1.02 (0.93–1.12)	0.69	
rs3135506	*APOA5*	1.04 (0.92–1.18)	0.52	
rs662799	*APOA5*	1.00 (0.88–1.12)	0.97	
Allele score		0.99 (0.96–1.01)	0.26	1.10 (1.08–1.12)
Q1 vs. Q2		0.95 (0.84–1.07)	0.39	1.19 (1.09–1.30)
Q1 vs. Q3		0.89 (0.79–1.01)	0.08	1.32 (1.19–1.46)
Q1 vs. Q4		0.90 (0.80–1.02)	0.11	1.37 (1.24–1.53)
Q1 vs. Q5		0.93 (0.82–1.06)	0.27	1.52 (1.34–1.72)

Q = quintile of weighted allele score. *Nearest gene information reported as in Kathiresan et al. (31), except for rs328, which is from Kathiresan et al. (34), and for rs3135506 and rs662799, which are from Pennacchio et al. (32). The sample sizes ranged from 10,378 to 10,574 for the individual SNP(s) vs. type 2 diabetes analyses. The number of individuals in the weighted allele score vs. type 2 diabetes analyses is 10,676 and ranged from 4,212 to 4,318 for quintiles of the allele score vs. type 2 diabetes analyses. The total number of individuals in the SNP(s) vs. type 2 diabetes analyses is greater than the corresponding SNP(s) vs. triglyceride analyses in Table 2 because the numbers in the latter were also restricted by the number of individuals with pretreatment triglyceride levels measured. The results for the SNP(s) vs. type 2 diabetes analyses were similar when we restricted the analyses to individuals with pretreatment triglycerides measured. †The point estimate of the expected OR for the allele score vs. type 2 diabetes was calculated by multiplying (in Fig. 1*a*) the effect size of the allele score vs. the triglyceride association by (in Fig. 1*b*) the OR of the triglyceride vs. type 2 diabetes association. For example, the expected type 2 diabetes OR for Q1 vs. Q2 was calculated by multiplying the effect size of the allele score vs. triglyceride association for the Q1 vs. Q2 comparison (0.17 SDs) by the natural log of the relevant triglyceride vs. type 2 diabetes OR (i.e., natural log of 2.9). The exponent of the answer from this multiplication is the expected type 2 diabetes OR for Q1 vs. Q2.

was a suggestive protective association (a 1-SD increase in [genetically influenced] circulating triglycerides was associated with an OR for type 2 diabetes of 0.61 [95% CI 0.45–0.83]; $P = 0.002$). There was strong evidence that the instrumental variable OR (0.61 [0.45–0.83]) and standard OR (2.68 [2.53–2.84]) estimates were different from each other ($P = 6 \times 10^{-21}$).

Instrumental variable estimates for fasting insulin, fasting glucose, HOMA-B, and HOMA-IR. Instrumental variable estimation gave strong evidence that genetically influenced circulating triglyceride levels do not have a causal effect on fasting insulin, fasting glucose, HOMA-B, or HOMA-IR (Table 3 and Fig. 3*C*). As found with the standard analyses described in the section "Observed association between triglyceride SNPs and fasting insulin, fasting glucose, HOMA-B, and HOMA-IR," there was evidence of heterogeneity in the instrumental variable analysis with fasting glucose as an outcome ($I^2 = 79.4\%$, $P = 0.002$), and removing the Go-DARTS study control subjects, who caused this heterogeneity (Fig. 3*C*), resulted in nominal evidence of the association of increased triglycerides with increased glucose levels ($P = 0.08$). For all four outcomes, the instrumental variable estimates from the meta-analyses were inconsistent with estimates observed from standard regression analyses (Table 3).

Effects of triglyceride SNPs on HDL, LDL, and total cholesterol and effects when including *GCKR* and *FADS1* SNPs. We found very similar results in the series of sensitivity analyses with some possible exceptions. First, using the weighted allele score containing the four SNPs with disproportionately greater effects on triglycerides relative to HDL, we observed a possible stronger protective effect of higher triglycerides on type 2 diabetes (instrumental variable analysis: OR per 1-SD increase in $\log_{10}$-triglycerides: 0.34 [95% CI 0.19–0.59]; $P = 0.0001$) (Supplementary Table 4). Second, including the *GCKR* and *FADS1* SNPs in the weighted allele model resulted in a possible protective association with fasting glucose levels compared with when these SNPs were not included (Supplementary Table 5). Third, adjusting for BMI resulted in a possible stronger protective effect of higher triglycerides on type 2 diabetes (0.35 [0.20–0.64]; $P = 0.001$), compared with when not adjusting for BMI (Supplementary Table 6).

DISCUSSION

Using a Mendelian randomization approach, our results show strong evidence that higher circulating triglyceride levels do not increase type 2 diabetes risk, fasting glucose, or fasting-based measures of insulin resistance. Our results are consistent with lifelong, raised circulating triglycerides conferring no net harm to the liver or β-cell. Our results suggest that alternative explanations are needed to explain the observational associations between raised triglyceride levels and diabetes and related traits. These explanations could include confounding factors or reverse-direction causal effects (i.e., type 2 diabetes and insulin resistance causing raised triglycerides). Other human genetic studies support the reverse-causation argument. For example, postreceptor defects in insulin resistance caused by *AKT* mutations result in increased hepatic lipogenesis and increased circulating triglycerides (8), and polymorphisms near the *IRS1* gene that are robustly associated with insulin resistance (42) also result in raised triglycerides (43) (both associations at conventional levels of genome-wide significance, $P < 5 \times 10^{-8}$).

TABLE 5
Associations of individual triglyceride SNPs and weighted allele score with fasting glucose and fasting insulin meta-analyzed across the four studies of nondiabetic individuals

		SNP(s) vs. fasting glucose				SNP(s) vs. fasting insulin			
SNP	Nearest gene*	Fasting glucose z score per allele (95% CI)	P	Heterogeneity P (I^2%)	Expected effect size in SDs (= a × b)†	Fasting glucose z score per allele (95% CI)	P	Heterogeneity P (I^2%)	Expected effect size in SDs (= a × b)†
rs2954029	*TRIB1*	0.02 (−0.01 to 0.05)‡	0.29	0.41 (0.0)		0.01 (−0.02 to 0.05)‡	0.45	0.22 (32.4)	
rs714052	*MLXIPL*	0.00 (−0.04 to 0.05)‡	0.86	0.43 (0.0)		−0.01 (−0.06 to 0.04)‡	0.59	0.75 (0.0)	
rs7557067	*APOB*	−0.04 (−0.07 to −0.00)‡	0.04	0.09 (53.2)		−0.03 (−0.07 to 0.01)‡	0.16	0.44 (0.0)	
rs17216525	*NCAN, CLIP2, PBX4*	0.01 (−0.05 to 0.06)‡	0.86	0.55 (0.0)		−0.02 (−0.08 to 0.04)‡	0.52	0.21 (33.3)	
rs10889353	*ANGPTL3*	−0.02 (−0.05 to 0.01)‡	0.14	0.47 (0.0)		0.02 (−0.02 to 0.05)‡	0.29	0.54 (0.0)	
rs7679	*PLTP*	−0.01 (−0.05 to 0.07)‡	0.55	0.10 (52.8)		0.02 (−0.03 to 0.06)‡	0.45	0.31 (15.4)	
rs7819412	*XKR6-AMAC1L2*	0.02 (−0.01 to 0.05)‡	0.11	0.73 (0.0)		0.05 (0.02 to 0.08)‡	0.004	0.68 (0.0)	
rs328	*LPL*	−0.03 (−0.08 to 0.02)‡	0.18	0.34 (10.9)		0.01 (−0.05 to 0.06)‡	0.85	0.09 (54.4)	
rs3135506	*APOA5*	−0.02 (−0.08 to 0.04)‡	0.55	0.09 (54.1)		−0.06 (−0.13 to 0.01)‡	0.114	0.75 (0.0)	
rs662799	*APOA5*	0.03 (−0.03 to 0.10)‡	0.30	0.64 (0.0)		0.03 (−0.04 to 0.10)‡	0.38	0.38 (2.8)	
Allele score		0.00 (−0.01 to 0.01)§	0.88	0.001 (80.9)	0.01 (0.01–0.02)	0.00 (−0.01 to 0.02)§	0.72	0.13 (47.6)	0.05 (0.04–0.05)
Q2 vs. Q1		−0.02 (−0.09 to 0.04)‖	0.41	0.28 (21.9)	0.03 (0.02–0.04)	0.02 (−0.05 to 0.10)‖	0.45	0.54 (0.0)	0.09 (0.06–0.11)
Q3 vs. Q1		−0.01 (−0.08 to 0.05)‖	0.74	0.22 (31.3)	0.04 (0.03–0.05)	0.04 (−0.03 to 0.16)‖	0.27	0.01 (73.3)	0.13 (0.12–0.14)
Q4 vs. Q1		−0.04 (−0.10 to 0.03)‖	0.27	0.20 (34.6)	0.05 (0.04–0.06)	−0.02 (−0.10 to 0.05)‖	0.53	0.22 (31.8)	0.16 (0.13–0.18)
Q5 vs. Q1		0.01 (−0.06 to 0.08)‖	0.74	0.004 (77.7)	0.07 (0.06–0.09)	0.04 (−0.03 to 0.11)‖	0.31	0.10 (52.7)	0.24 (0.21–0.27)

Q = quintile of weighted allele score. *Nearest gene information reported as in Kathiresan et al. (31), except for rs328, which is from Kathiresan et al. (34), and for rs3135506 and rs662799, which are from Pennacchio et al. (32). †The point estimate of the expected effect size for the allele score vs. outcome was calculated by multiplying (Fig. 1*a*) the effect size of the allele score vs. triglyceride association by (Fig. 1*b*) the effect size of the relevant triglyceride vs. outcome association. For example, the expected effect size for the allele score vs. fasting glucose was calculated by multiplying the effect size of the allele score vs. triglyceride association (0.12 SDs) by the effect size of the triglyceride vs. fasting glucose association (0.12 SDs). The effect sizes reported are ‡change in fasting glucose/fasting insulin z score per triglyceride-raising allele for individual SNPs, §change in fasting glucose/fasting insulin z score per unit increase in weighted allele score, or ‖difference in fasting glucose/fasting insulin z score between the relevant quintiles of the weighted allele score. The sample sizes for allele score vs. outcome analyses were 8,040 and 6,544 for fasting glucose and fasting insulin, respectively. For the quintiles of allele score vs. outcomes, the sample sizes ranged from 3,197 to 3,216 and from 2,633 to 2,643 for fasting glucose and fasting insulin, respectively.

There are several strengths and limitations to our approach. The main strength is that we used genetic variants to test a complex relationship between metabolic traits. Because genetic variation is randomly sorted at meiosis, associations between SNPs and metabolic traits are unlikely to be biased, confounded, or influenced by disease processes. Furthermore, the effects of the genetic variants we have used are likely to reflect lifelong exposure to altered circulating triglycerides. In contrast, it is extremely difficult to disentangle likely causal directions between correlated human phenotypes using nongenetic approaches (38), and this is especially true for associations between metabolic factors such as lipid levels, diabetes, and insulin resistance (7). The second strength of our study is the statistical power. Because we used 10 common variants, our weighted allele score model compared large numbers of people with large differences in genetically influenced circulating triglyceride levels; for example, 20% of individuals carrying the most triglyceride-raising alleles had circulating levels 0.59 SDs higher than the 20% carrying the fewest. We therefore had very good power to see an effect of triglyceride variants on related metabolic traits if such a relationship existed (for example >80% power at $P = 0.05$ if circulating triglycerides 0.59 SDs higher than a baseline group resulted in a type 2 diabetes OR of 1.12). The third strength is that we used 10 variants that are likely to influence circulating triglyceride levels in a variety of ways. Although genome-wide association studies do not identify the causal gene involved, variants in or near *LPL* and *ANGPTL3* are likely to influence lipoprotein lipase function, the key enzyme located in capillary surfaces that hydrolyses triglycerides to release fatty acids (44,45). Variants in *APOA5* are among those with the strongest effects on circulating triglycerides and are likely to function through a variety of mechanisms, including reducing liver production of triglycerides (46,47). Variants near *APOB* are most likely to affect triglyceride clearance from the liver, and the variant at the *PLTP* locus is associated with altered *PLTP* expression in human liver samples, suggesting that it operates in the liver (31). Our data therefore suggest that the lack of association between circulating triglycerides, type 2 diabetes, and related outcomes is not dependent on the particular mechanism that alters triglyceride levels. A fourth strength of our study is that our results for continuous traits are consistent across four studies of different characteristics, including mean age ranges between 33.9 and 68.8 years, mean BMIs between 25.52 and 27.24 kg/m^2, and different ratios of male and female subjects. The exception is fasting glucose, to which the Go-DARTS study contributes significant heterogeneity between studies, and the results are consistent with a small effect of triglycerides on fasting glucose levels in the remaining three studies.

There are several limitations to our study. Most importantly, we are testing circulating, not intracellular, triglycerides. We have not tested the role of triglycerides in the liver, and fasting insulin (and HOMA-IR) is primarily a measure of hepatic insulin resistance rather than muscle insulin resistance. Several of the gene variants are likely to operate in the liver by increasing the clearance of triglycerides into the circulation, which could be consistent with a lack of effect of these variants on hepatic-based measures of insulin resistance. A net effect of the triglyceride-raising alleles on increased clearance of triglycerides from the liver could also explain the suggestive protective association between increased (genetically influenced) circulating triglycerides and reduced risk of type 2 diabetes in the instrumental variable analysis. However, this association was not reflected by a protective association between triglyceride-raising alleles and hepatic measures of insulin resistance and could be attributed to chance. It will be important to test the association between triglyceride variants and oral glucose tolerance test–based or muscle-based measures of insulin resistance, such as those based on hyperinsulinemic-euglycemic clamps. Therefore, our results do not necessarily provide evidence against the lipotoxicity hypothesis, which states that raised triglyceride levels contribute to whole-body insulin resistance. In contrast, our results provide stronger evidence against the lipotoxicity hypothesis, in that raised circulating triglyceride levels contribute to altered β-cell function and type 2 diabetes. A second limitation is that we have not tested the effects of raised circulating triglyceride levels alone but rather a mixture of raised circulating triglycerides and, to a lesser extent, raised LDL and total cholesterol and lower HDL cholesterol. However, an analysis using just the four variants with disproportionate effects on circulating triglyceride levels relative to HDL cholesterol provided similar results. With the identification of an increasing number of genetic variants related to lipid fractions, it will be possible to produce multiple allele score instruments, which would allow a demonstration of a lack of pleiotropy in generating the observed findings. Finally, the 10 SNPs used only account for 3–5% of the phenotypic variation in circulating triglyceride levels. We have therefore not tested the full spectrum of genetically influenced triglyceride levels.

Further Mendelian randomization studies will be needed to test the role of circulating FFAs, which may be more critical to reduced β-cell function than triglycerides (48). We excluded from our main analysis the common variant near the *FADS1* gene because this variant is most strongly associated with polyunsaturated fatty acids (49). The *FADS1* variant is also associated with fasting-based measures of insulin secretion, such as fasting glucose and HOMA-B, and to a lesser extent type 2 diabetes (29), suggesting that FFAs could have a causal role in diabetes. Additional genetic studies are needed to assess the role of FFAs and different types of FFAs in insulin resistance and secretion.

In conclusion, we have performed a powerful Mendelian randomization analysis of circulating triglyceride levels. Our data provide evidence that genetically influenced raised circulating triglyceride levels do not increase the risk of type 2 diabetes and related metabolic traits.

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