Obesity Partially Mediates the Diabetogenic Effect of Lowering LDL Cholesterol

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OBJECTIVE

LDL cholesterol (LDLc)-lowering drugs modestly increase body weight and type 2 diabetes risk, but the extent to which the diabetogenic effect of lowering LDLc is mediated through increased BMI is unknown.

RESEARCH DESIGN AND METHODS

We conducted summary-level univariable and multivariable Mendelian randomization (MR) analyses in 921,908 participants to investigate the effect of lowering LDLc on type 2 diabetes risk and the proportion of this effect mediated through BMI. We used data from 92,532 participants from 14 observational studies to replicate findings in individual-level MR analyses.

RESULTS

A 1-SD decrease in genetically predicted LDLc was associated with increased type 2 diabetes odds (odds ratio [OR] 1.12 [95% CI 1.01, 1.24]) and BMI ($\beta = 0.07$ SD units [95% CI 0.02, 0.12]) in univariable MR analyses. The multivariable MR analysis showed evidence of an indirect effect of lowering LDLc on type 2 diabetes through BMI (OR 1.04 [95% CI 1.01, 1.08]) with a proportion mediated of 38% of the total effect ($P = 0.03$). Total and indirect effect estimates were similar across a number of sensitivity analyses. Individual-level MR analyses confirmed the indirect effect of lowering LDLc on type 2 diabetes through BMI with an estimated proportion mediated of 8% ($P = 0.04$).

CONCLUSIONS

These findings suggest that the diabetogenic effect attributed to lowering LDLc is partially mediated through increased BMI. Our results could help advance understanding of adipose tissue and lipids in type 2 diabetes pathophysiology and inform strategies to reduce diabetes risk among individuals taking LDLc-lowering medications.

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Emerging data from large-scale randomized clinical trials have shown that LDL cholesterol (LDLc)-lowering drugs influence glycemic control in addition to their hypolipidemic and cardioprotective effects (1–3). This evidence is supported by data showing that naturally occurring genetic variation in molecular targets of LDLc-lowering drugs, such as genetic variants in or near *HMGCR*, *NCP1L1*, and *PCSK9*, are associated with impaired glycemic control and higher risk of type 2 diabetes (3–6). In absolute terms, such risk represents one additional case per 255 patients taking lipid-lowering drugs for 4 years (1).

Preliminary studies have also provided evidence that lowering LDLc is associated with weight gain (3–6). In a meta-analysis of lipid-lowering clinical trials, LDLc-lowering therapy increased body weight by 0.24 kg after 4 years of follow-up (3). Furthermore, in a combined analysis of genetic studies, each additional LDLc-lowering risk allele at *HMGCR* gene, which reduced LDLc by 0.06 mmol/L (95% CI 0.05, 0.07), was associated with 0.30 kg/m^2 higher BMI (3). Similar observations have been reported for variation in or near other lipid-lowering drug targets such as *PCSK9* (5,6). This suggests that the increased type 2 diabetes risk observed in lipid-lowering trials and genetic studies might be in part mediated by weight gain, but no studies have tested this hypothesis to date.

In this study, we leveraged human genetic data to test the hypothesis that the diabetogenic effect of LDLc lowering is mediated through increased BMI. We used summary-level data from three large-scale genetic studies including 921,908 participants of European descent to conduct univariable and multivariable Mendelian randomization (MR) analyses. Then, we implemented individual-level MR analyses to replicate the findings in 92,532 participants from 14 observational studies.

RESEARCH DESIGN AND METHODS

Study Design

We conducted summary-level univariable and multivariable MR analyses to assess the extent to which the diabetogenic effect of LDLc lowering is mediated through BMI. MR is a methodological approach that uses human genetic variation associated with modifiable exposures as instrumental variables to test the causal effect of a risk factor on a disease or health-related outcome. With MR, a genetic variant serves as a valid instrument if certain assumptions hold, including that the genetic variant is associated with the exposure of interest, there are no common causes of genotype and health outcome, and the genetic variant affects the outcome only through their effect on the risk factor of interest (7). Summary-level MR analyses were conducted with use of data from large-scale genome-wide association studies (GWAS) for LDLc from UK Biobank (8), BMI from Genetic Investigation of ANthropometric Traits (GIANT) (9), and type 2 diabetes from DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) (10). Summary-level MR analyses were complemented with the analysis of individual-level data in 92,532 participants from 14 observational studies within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (11). Figure 1 conceptually depicts

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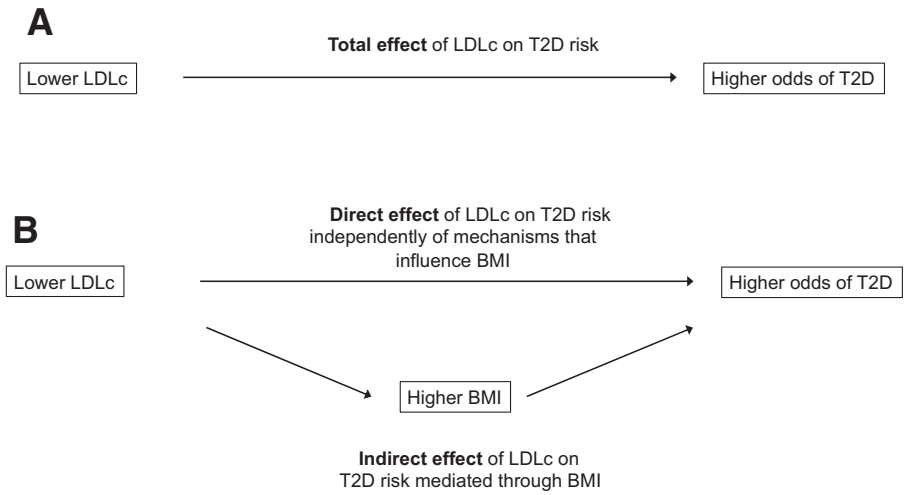

Figure 1—Direct acyclic graph to illustrate total, direct, and indirect effects of LDLc on type 2 diabetes risk. Directed acyclic graphs demonstrating the hypothesized direction for the total effect of lower LDLc on increased odds of type 2 diabetes (T2D) (*A*) and the hypothesized direction for the effect of lower LDL-c on increased BMI (*B*), which may partially mediate the effect of lowering LDLc on T2D risk.

our approach, and Tables 1 and 2 summarize the studies included in each analysis.

Data Sources

We obtained summary statistics from GWAS for each respective phenotype. For circulating lipid traits, we obtained data based on 440,546 UK Biobank participants of European ancestry (8). These traits included LDLc, HDL cholesterol (HDLc), and triglycerides. We did not exclude participants already on statins in UK Biobank because the exclusion of these participants' results would be prone to collider bias when these genetic variants are used as genetic instruments in MR (12). Lipid traits were rank normalized such that the GWAS effect sizes are in SD units, corresponding to 0.87 mmol/L (8). Covariate adjustments in these GWAS included age, sex, and genotyping array, and population stratification was addressed through the use of linear mixed models (13). To identify genetic instrumental variables for LDLc, we first selected variants associated with LDLc at genome-wide significance ($P < 5 \times 10^{-8}$) that were also available in the type 2 diabetes GWAS data set. These variants were then clumped with use of a pairwise linkage disequilibrium (LD) cutoff of $r^2 < 0.001$ within a 1-Mb clumping window, estimated with data from the 1000 Genomes European as a reference panel. Palindromic variants were excluded. With this procedure we identified 232 genetic instruments (Supplementary Table 1).

For BMI, we obtained genetic association estimates for BMI from the GIANT consortium's 2015 GWAS meta-analysis of 322,154 participants of European descent (9). BMI was rank normalized such that the GWAS effect sizes are in SD units (corresponding to $\sim$4.7 kg/m^2). Models were adjusted for age, age squared, and study-specific covariates (including principal components to adjust for population stratification). The meta-analysis of study-specific GWAS was corrected by double genomic control to account for population stratification. Using the same variant selection procedure detailed above, we identified 75 variants to use as instrumental variables for BMI (Supplementary Table 2).

Association estimates for type 2 diabetes were obtained from publicly available genetic association estimates from DIAGRAM GWAS meta-analysis of 26,676 T2D case and 132,532 control subjects (10). Statistical adjustment in this GWAS included age, sex, and principal components of ancestry.

For replication of summary-level MR findings, we included data from 14 cohorts within the CHARGE consortium to conduct individual-level MR analyses. A total of 92,532 individuals ($n = 12,073$ prevalent type 2 diabetes cases) with complete genotype and phenotype data and without prevalent cardiovascular disease, including coronary heart disease, cerebrovascular disease, and peripheral artery disease, were included in these analyses. Detailed characteristics of the participating cohorts and study participants are shown in Table 2, Supplementary Table 3, and Supplementary Appendix 1. All study participants provided written informed consent to participate in genetic studies, and ethics approval to conduct this study was obtained from local research ethics committees.

For individual-level MR analyses we elaborated a prespecified protocol including information, such as definitions of

Table 1—Characteristics of GWAS included in summary-level MR analyses

Trait/phenotype	GWAS consortium	Ethnicity	Sample size (total or case/control subjects)	Unit of measure	PMID
LDLc	UK Biobank	European	440,546	1 SD (mmol/L)	32203549
BMI	GIANT	European	322,154	1 SD (kg/m^2)	25673413
T2D	DIAGRAM	European	26,676 / 132,532	Log-odds	28566273

GWAS data sets included in summary-level MR analyses. PMID, PubMed identifier; T2D, type 2 diabetes.

Table 2—Characteristics of the cohorts included in individual-level MR analyses

	Abbreviation	Country	Sample size	T2D cases
Coronary Artery Risk Development Study in Young Adults	CARDIA	U.S.	1,715	253
Cardiovascular Health Study	CHS	U.S.	4,276	448
Danish General Suburban Population Study	GESUS	Denmark	7,120	321
European Prospective Investigation into Cancer and Nutrition-Potsdam study	EPIC-Potsdam	Germany	2,316	93
Family Heart Study	FamHS	U.S.	2,353	256
Framingham Heart Study	FHS	U.S.	5,368	601
Hispanic Community Health Study / Study of Latinos	HCHS/SOL	U.S.	11,822	2,271
Jackson Heart Study	JHS	U.S.	2,992	999
Johns Hopkins Genetic Study of Atherosclerosis Risk	GeneSTAR	U.S.	2,526	379
Malmö Diet and Cancer–Cardiovascular Cohort	MDC-CC	Sweden	4,764	830
Mass General Brigham Biobank	MGBB	U.S.	13,925	1,806
Multi-Ethnic Study of Atherosclerosis	MESA	U.S.	4,912	1,064
Rotterdam Study	RS	The Netherlands	7,686	842
Women's Genome Health Study	WGHS	U.S.	20,757	1,910
Total			92,532	12,073

Shown for each participating cohort are the country of origin, the available sample size with genetic and exposure information, and the number of individuals T2D that were included.

exposures, outcomes, and covariates, and statistical analysis plan prior to data analysis. The document that was distributed to each participating study can be found in Supplementary Appendix 2. For individual-level MR analyses, ascertainment of type 2 diabetes was defined on the basis of fasting or nonfasting glucose determinations or treatment with either insulin or hypoglycemic agents or reviewing multiple sources of evidence, including linkage to primary care registers and hospital admissions. LDLc was estimated with the Friedewald formula (14) or directly measured with enzymatic assays. BMI was calculated as weight in kilograms divided by the square of the height in meters.

Statistical Analysis

We performed summary-level univariable MR analyses to investigate the total, indirect, and direct effects of LDLc on type 2 diabetes (Fig. 1). The total effect is defined as the net effect of genetically predicted LDLc on type 2 diabetes irrespective of mechanism and was estimated with 232 LDLc genetic instruments. The indirect effect is defined as the effect of genetically predicted LDLc on type 2 diabetes that is mediated through BMI. The indirect effect was calculated with the product of coefficients method (15), in which we multiplied the MR estimate for the effect of LDLc on BMI and the MR estimate for the effect of BMI on type 2 diabetes. To test the null hypothesis of no mediation through BMI, we calculated CIs for the indirect effect using the previously described Monte Carlo method (16). We used the propagation of error method to derive a *P* value for the indirect effect (17). We also calculated the proportion of the mediated effect by dividing the indirect effect by the total effect. The direct effect is defined as the association of genetically predicted LDLc on type 2 diabetes through mechanisms independent of mediation. To estimate the direct effect, we used multivariable MR. For multivariable MR analyses, we again used variants from the univariable analysis after undertaking further LD clumping to account for correlation between LDLc and BMI genetic instruments. A total of 259 variants were used as instrumental variables in multivariable MR (Supplementary Table 4).

We assessed LDLc instrument strength by deriving the *F* statistic based on the proportion of variance in the phenotype explained by the genetic variants, sample size, and number of instruments (18). The overall effect sizes on type 2 diabetes were reported as odds ratios (ORs) and 95% CIs of OR per 1SD decrease in genetically predicted LDLc, which corresponds to 0.87 mmol/L (8). In the figures, we used β-coefficients to report estimated effect sizes due to the inclusion of binary and continuous outcomes in the same figure, but in the main text we elected to provide OR (=exp(β)) for binary outcomes, as it is easier to interpret than the β-coefficients. Heterogeneity was examined with Cochran's *Q* statistic (19). Summary-level MR analyses were conducted with the inverse variance–weighted method implemented in the TwoSampleMR package v4.26 (20).

We performed sensitivity analyses that are more robust than the inverse variance–weighted method to certain forms of pleiotropy, including the weighted median (21), MR-Egger (22), and MR pleiotropy residual sum and outlier (MR-PRESSO) (23). Given the strong effect of genetic variation in *FTO* on BMI, we performed analyses excluding lead variants in this locus. In a separate sensitivity analysis to investigate the extent to which our results were affected by pleiotropic effects of LDLc genetic variants on other lipids, we conducted multivariable MR analyses to account for pleiotropic effects of LDLc genetic variants on HDLc and triglycerides. Because reverse causal effect of

mediator on exposure or outcome on mediator may bias mediation estimates (16), we used MR-Steiger to filter out genetic instruments that explained more of the variance in the outcome trait than in the exposure (24). We also investigated whether LDLc-lowering alleles in or near genes encoding molecular targets of lipid-lowering therapy (*NPC1L1*, *HMGCR*, *PCSK9*, and *LDLR*) were associated with increased odds of type 2 diabetes and BMI. For these analyses we included all variants within 100 kb on either side of each lipid-lowering therapy target gene that were associated with LDLc at a genome-wide level of significance and that were in a pairwise LD cutoff of $r^2 < 0.001$ within a 1-Mb clumping window.

Individual-level MR analyses were conducted separately in studies from the CHARGE Consortium. We estimated the causal effect of the exposure on the outcome using two-stage least squares regression. In the first stage, we respectively regressed each exposure of interest on 232 LDLc and 75 BMI genetic instruments and obtained their predicted values, respectively. The genetic instruments were encoded into dosage according to the number of LDLc or BMI increasing alleles from the respective GWAS, and variants were included separately rather than aggregated in a polygenic score. In the second stage, logistic and linear regression models were fitted with adjustment for age, sex, the first five ancestry-derived principal components, and cohort-specific covariates. To obtain the total effect of LDLc on type 2 diabetes we performed logistic regression with type 2 diabetes as the outcome and genetic predicted LDLc as exposure. We estimated indirect effects by taking the product of predicted LDLc effect on BMI and predicted BMI effect on type 2 diabetes. We used multivariable MR to estimate direct effect. The predicted LDLc was first obtained from the clumped list of 259 genetic instruments for LDLc and BMI. Then, we conducted logistic regression with type 2 diabetes as the outcome on predicted LDLc adjusting for age, sex, the first five ancestry-derived principal components, and cohort-specific covariates. We combined the estimated effects from participating cohorts using fixed-effects meta-analysis.

RESULTS

We conducted summary and individual-level MR analyses to investigate the extent to which BMI partially mediated the effect of lowering LDLc on type 2 diabetes odds (Fig. 1). The 232 genetic instruments for LDLc explained 7% of the variance in LDLc, with a mean *F* statistic of 142, indicating no evidence of weak genetic instruments.

A 1-SD reduction in genetically predicted LDLc increased the odds of type 2 diabetes by 12% (95% CI 1.01, 1.24; *Q* test $P < 0.001$) (Fig. 2*A*). To calculate the effect of lowering LDLc on type 2 diabetes mediated through BMI, we first tested for an association of genetically predicted LDLc with BMI and showed that 1 SD reduction in genetically predicted LDLc increased BMI by 0.07 SD units (95% CI 0.02, 0.12; *Q* test $P < 0.001$) (Fig. 2*A*). Next, we tested for an association of genetically predicted BMI with type 2 diabetes and found that 1 SD increase in genetically predicted BMI had an OR for type 2 diabetes of 2.05 (95% CI 1.45, 2.92; *Q* test $P < 0.001$) (Fig. 2*A*). For the effect of LDLc on odds of type 2 diabetes mediated through BMI, we estimated an OR of 1.05 (95% CI 1.01, 1.10) (Fig. 2*A*), and the calculated proportion mediated was 44% of the total effect ($P = 0.03$) (Fig. 2*A*). In multivariable MR, we observed less evidence of a direct effect of lowering LDLc on odds of type 2 diabetes (OR 1.12 [95% CI 0.96, 1.31]) (Fig. 2*A*) and an OR of 1.04 (95% CI 1.01, 1.08) for the indirect effect (Fig. 2*A*) with a proportion mediated of 38% of the total effect ($P = 0.03$) (Fig. 2*A*).

We performed several sensitivity analyses. We first leveraged methods that relax the MR assumption of no unbalanced horizontal pleiotropy and observed largely consistent estimates for the total effect of lowering LDLc on the odds of type 2 diabetes and BMI (Supplementary Table 5). Results were similar after exclusion of genetic instruments in the *FTO* locus (Supplementary Table 5). We also investigated whether our results were affected by pleiotropic effects of LDLc genetic variants on other lipid traits. This analysis provided consistent estimates on the total effect of lowering LDLc on type 2 diabetes odds after pleiotropic effects on triglycerides and HDLc (OR 1.18 [95% CI 1.05, 1.33]) (Supplementary Table 5) were accounted for. When analyzed within LDLc drug targets, the diabetogenic effect of lowering LDLc was particularly evident for genetic variation in or near *NPC1L1* and *PCSK9* (OR 4.44 [95% CI 1.84, 10.70] and OR 1.32 [95% CI 1.01, 1.73] per 1 SD reduction in genetically driven LDLc, respectively) (Supplementary Fig. 1). LDLc-lowering alleles at *HMGCR* were primarily associated with increased BMI by 0.29 SD units (95% CI 0.20, 0.39) (Supplementary Fig. 1). Further, in an analysis to investigate potential reverse causal effects, we observed results largely consistent with those of our primary analysis. In this sensitivity analysis, for the indirect effect of LDLc on type 2 diabetes risk there was an OR of 1.04 (95% CI 1.01, 1.07) and the calculated proportion mediated through BMI was 39% of the total effect ($P = 0.003$) (Supplementary Table 6).

Using individual-level data from 92,532 participants, we showed that 1 SD reduction in genetically predicted LDLc increased the odds of type 2 diabetes by 20% (95% CI 1.12, 1.27) (Fig. 2*B*). In an analysis to investigate the effect of lowering LDLc on BMI, we showed that 1 SD reduction in genetically driven LDLc increased BMI by 0.02 SD units (95% CI 0.00, 0.04) (Fig. 2*B*). A 1-SD increase in genetically predicted BMI increased the odds of type 2 diabetes by 97% (95% CI 1.92, 2.03) (Fig. 2*B*). We estimated an OR for the effect of LDLc on type 2 diabetes risk mediated through BMI of 1.01 (95% CI 1.00, 1.03), and the calculated proportion mediated was 8% of the total effect ($P = 0.04$) (Fig. 2*B*). Similar to summary-level MR, we observed less evidence of a direct effect of lowering LDLc on type 2 diabetes odds in individual-level MR (OR 1.17 [95% CI 0.99, 1.39]) (Fig. 2*B*). Cohort-specific estimates are provided in Supplementary Figs. 2 and 4.

CONCLUSIONS

Results from this MR study with use of large-scale human genetic data sets support the observation that lowering LDLc has a causal effect on risk of type 2 diabetes and provide consistent evidence that the diabetogenic effects of lowering LDLc are in part mediated through increased BMI. These results could help prioritize investigation of weight gain prevention to mitigate type 2 diabetes risk among individuals taking

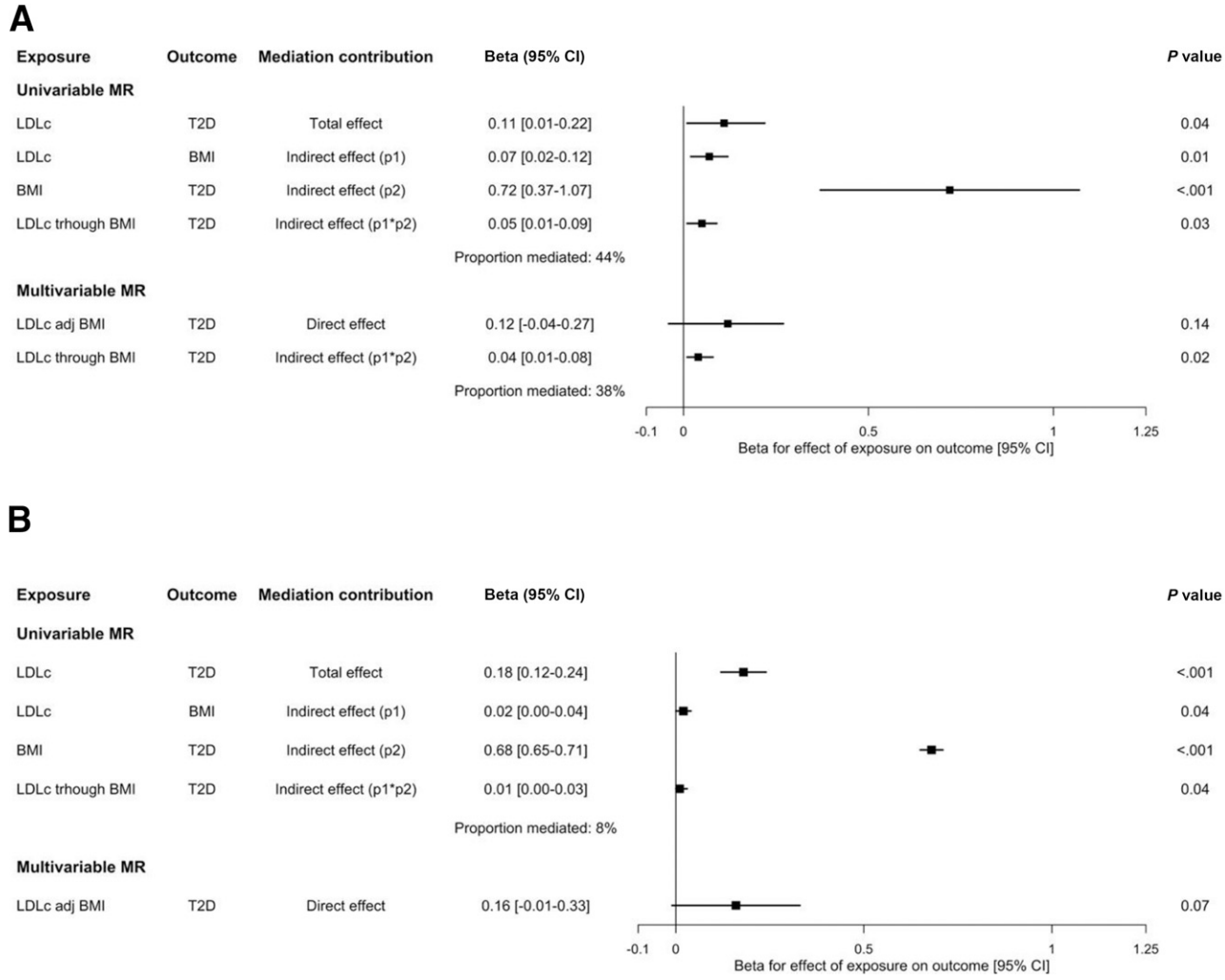

Figure 2—MR estimates for the total, indirect, and direct effect of a genetically predicted low LDLc on type 2 diabetes risk. Forest plot of univariable and multivariable MR estimates for the total, indirect, and direct effect of a genetically driven low LDLc on type 2 diabetes risk from summary-level (*A*) and individual-level (*B*) analyses. The indirect effect was calculated with the products of the coefficient method (RESEARCH DESIGN AND METHODS). The direct effect was obtained with multivariable MR (RESEARCH DESIGN AND METHODS). Estimates for genetically predicted LDLc analyses were oriented to reflect the effect of a 1-SD decrease in LDLc on the outcome. We used β-coefficients to report estimated effect sizes in the figure due to the inclusion of binary and continuous outcomes, but in the main text we elected to provide ORs (=exp(β)) for binary outcomes, as these are easier to interpret than the β-coefficients. LDLc adj BMI, LDLc adjusted for BMI; T2D, type 2 diabetes.

LDLc-lowering medications and inform future studies to provide insight into molecular mechanisms linking adipose tissue and lipids in type 2 diabetes pathophysiology.

The effect of genetically predicted LDLc on type 2 diabetes is aligned with previous evidence from both meta-analysis of randomized controlled trials and genetic studies showing that drugs designed to reduce LDLc are associated with impaired insulin sensitivity and new-onset type 2 diabetes (1–6). In support of these observations, individuals with high LDLc levels due to familial hypercholesterolemia appear to have a lower prevalence of diabetes than unaffected relatives (25). However, data from Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER), a randomized, placebo-controlled trial of subcutaneous injections of the anti-PCSK9 monoclonal antibody evolocumab, showed no evidence of an association between pharmacological PCSK9 inhibition and incidence of new-onset diabetes or glycemic alterations (26). While FOURIER was adequately powered to detect the effect sizes for diabetes risk identified in genetic studies, the many differences between MR and clinical trials in terms of duration, scale, and timing might explain the discrepant results. For example, while MR estimates from our study can be interpreted in the context of lifelong exposure to reduced LDLc in the general population, FOURIER investigated pharmacological PCSK9 inhibition over ∼2 years of follow-up in patients with atherosclerotic disease who were on statin therapy. Through leveraging of the most recent genetic associations for LDLc in MR analyses, our results further support that the diabetogenic effect of lowering LDLc is likely attributable to processes related to modification of LDLc per se rather than to pleiotropic effects of lipid-lowering medications.

Our study adds to knowledge by formally investigating whether increased BMI mediates the effect of lowering LDLc on type 2 diabetes risk. Previous meta-analyses of lipid-lowering trials have described a subtle increase in weight

caused by lipid-lowering drugs (3). Further, genetic variants associated with lower circulating LDLc have also associated with modest increase in BMI (3–6). However, none of previous studies could establish whether the diabetogenic effect of LDLc lowering operates through increased BMI. By conducting mediation analysis in the context of MR, our study provides evidence of an indirect effect of LDLc on type 2 diabetes risk through BMI, suggesting that molecular pathways for lower circulating LDLc converge into mechanisms related to higher BMI. However, reverse causation might still exist even that we observed little evidence of such effects in a sensitivity analysis excluding genetic instruments more strongly linked to the outcome than the exposure. In addition, the potential diabetogenic effect of lowering LDLc mediated through BMI could be explained by changes in diet among people taking hypolipemic medications as documented in a previous study in which caloric intake increased by ∼10% (95% CI 1.8, 18.1) from 1999–2000 to 2009–2010 among individuals taking statins (27). Findings from our study, in the context of MR, suggest that BMI is a causal mediator of the diabetogenic effect of lowering LDLc and that this effect is less likely to be confounded by other measured or unmeasured factors such as increased caloric intake. Our summary-level MR analysis supports that ∼40% of the effect of lowering LDLc on increased type 2 diabetes risk is mediated through BMI, but this estimate was attenuated to 8% in the individual-level MR setting. Lack of independence between gene-exposure and gene-outcome estimates in the presence of confounding in the individual-level MR setting might explain why the proportion mediated was attenuated (28).

Results from this study may inform further studies to better understand pathophysiological processes leading to dyslipidemia and impaired glucose metabolism. For example, new studies to investigate adipocyte physiology in the context of lowering LDLc might add to previous experimental observations showing that lowering LDLc impairs adipocyte maturation (29), differentiation (30), and adipokine secretory profile (31,32). In addition, previous experimental studies have suggested that methyl-β-cyclodextrin–mediated cholesterol depletion of 3T3-L1 adipocytes results in defective glucose uptake and oxidation, diminished GLUT-4 expression, and impaired insulin signaling (33) and that increased intracellular cholesterol produces islet cell dysfunction with reduced insulin secretion and cell proliferation (34). Our results support further investigations into the cross talk between adipose tissue and the liver and are aligned with data showing that genes involved in intracellular lipid and cholesterol transport, processes that occur primarily in adipocytes but are closely linked to liver metabolism, are responsible for the inverse effect on LDLc and blood glucose (35). A recent study has also shown that genetic variants with opposite effects on LDLc and type 2 diabetes are mainly involved in lipogenesis, hepatic fat uptake, and insulin secretion and action (36). Of note, several of the identified loci with opposite effects on LDLc and type 2 diabetes were also associated with BMI, including sortilin 1 (*SORT1*). *SORT1* is highly expressed in adipocytes and hepatocytes, and the sortilin gene product facilitates the formation and export of VLDL from the liver (37). Taken together, these results highlight the relevance of adipose tissue and liver in the diabetogenic effect of lowering LDLc and support further investigations to better understand molecular mechanisms by which lowering LDLc might impact adipocyte function, lipid metabolism, and dysglycemia.

The implication that increased BMI partially mediates the effect of lowering LDLc and type 2 diabetes risk could help inform clinical interventions to mitigate the diabetogenic effects of lipid-lowering medications. For example, lipid-lowering strategies that promote adipose tissue expandability might have relevant implications for mitigating the dysglycemic effects of lowering LDLc. Previous evidence suggests that the *ANGPTL4* p.Glu40Lys loss-of-function variant is associated with directionality-consistent effects on type 2 diabetes and coronary artery disease and that their cardioprotective benefits are consistent across the population distribution of LDLc-lowering alleles (38). While the cardioprotective benefit of lipid-lowering medications vastly outweighs the harm from increased type 2 diabetes risk (39), findings from this and other studies might have relevant clinical implications, as they suggest it might be prudent to monitor body weight and glycemic status after initiation of lipid-lowering medications, especially among those at high risk for type 2 diabetes.

While MR is more robust to confounding and measurement error relative to conventional observational methods (7), our results may still be biased by pleiotropic or bidirectional effects of the variants modeled as instrumental variables. Although such bias cannot be entirely excluded, it is reassuring that we obtained similar estimates in several sensitivity MR methods, each of which make different assumptions concerning the presence of pleiotropic or bidirectional variants. Sample overlap in the context of MR means that estimated effect sizes for variants associated with the exposure and the outcome are partly coming from the same participants. Sample overlap might bias the causal effect estimate induced by environmental confounding. A recent study using simulation and real data has shown that the magnitude of sample overlap bias is likely to be small and that sample overlap usually leads to an underestimation of the true causal effect (40), which means that the contributions of LDL-lowering therapies to obesity and type 2 diabetes risk are likely higher than we report here. Also, summary-level analyses were performed using data from European populations, while ancestry-diverse populations were included in the individual-level MR analysis. For alleviation of potential issues related to low transferability of genetic associations identified in GWAS that mostly included European descent participants to other populations, our genetic instruments in individual-level MR analyses were included separately without weighting rather than aggregated in a polygenic score with European-derived weights. Nonetheless, generalizability to other ancestry groups might be uncertain.

In conclusion, our findings support that elevated BMI partially mediates the diabetogenic effects observed with lowering LDLc. Further exploration of this mechanism may yield insights into adipose tissue and type 2 diabetes pathophysiology, and targeted weight control strategies may be investigated to mitigate the increased risk of type 2 diabetes among

individuals taking LDLc-lowering therapies.

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