



Estimating the Effect of Liver and Pancreas Volume and Fat Content on Risk of Diabetes: A Mendelian Randomization Study

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OBJECTIVE

Fat content and volume of liver and pancreas are associated with risk of diabetes in observational studies; whether these associations are causal is unknown. We conducted a Mendelian randomization (MR) study to examine causality of such associations.

RESEARCH DESIGN AND METHODS

We used genetic variants associated ($P < 5 \times 10^{-8}$) with the exposures (liver and pancreas volume and fat content) using MRI scans of UK Biobank participants ($n = 32,859$). We obtained summary-level data for risk of type 1 (9,358 cases) and type 2 (55,005 cases) diabetes from the largest available genome-wide association studies. We performed inverse-variance weighted MR as main analysis and several sensitivity analyses to assess pleiotropy and to exclude variants with potential pleiotropic effects.

RESULTS

Observationally, liver fat and volume were associated with type 2 diabetes (odds ratio per 1 SD higher exposure 2.16 [2.02, 2.31] and 2.11 [1.96, 2.27], respectively). Pancreatic fat was associated with type 2 diabetes (1.42 [1.34, 1.51]) but not type 1 diabetes, and pancreas volume was negatively associated with type 1 diabetes (0.42 [0.36, 0.48]) and type 2 diabetes (0.73 [0.68, 0.78]). MR analysis provided evidence only for a causal role of liver fat and pancreas volume in risk of type 2 diabetes (1.27 [1.08, 1.49] or 27% increased risk and 0.76 [0.62, 0.94] or 24% decreased risk per 1SD, respectively) and no causal associations with type 1 diabetes.

CONCLUSIONS

Our findings assist in understanding the causal role of ectopic fat in the liver and pancreas and of organ volume in the pathophysiology of type 1 and type 2 diabetes.

The pancreas and the liver play key roles in the pathogenicity of both type 1 and type 2 diabetes in the context of β -cell dysfunction (1) and insulin resistance (2). Studies using autopsies, ultrasound, computed tomography, and MRI have provided three main observations in comparisons of the levels of liver and pancreas fat deposition and volume between individuals with and without diabetes. First, individuals with type 1 (3) and type 2 (4) diabetes have smaller pancreases compared

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with healthy control subjects. Second, pancreatic fat is higher in people with type 2 diabetes compared with age-matched control subjects (5) and is negatively associated with insulin secretion (6). Third, accumulation of fat in the liver has been linked to resistance to insulin-mediated gluconeogenesis and development of type 2 diabetes (7).

These observations might be confounded by some unknown factors and therefore may not concur with the causal nature of the associations. These limitations can be avoided by using Mendelian randomization (MR) analysis given the assumptions are met. MR is a method that uses genetic variants reliably associated with exposures of interest to estimate a nonconfounded causal association between the exposure (e.g., pancreatic fat) and an outcome (e.g., type 2 diabetes) (8). Since allelic variants remain stable over time, their lifetime effects on the exposure levels precede the outcome and limit bias from reverse causation.

Understanding the exact role of liver and pancreas in diabetes risk may be helpful to develop more effective prevention, prediction, and treatment or to supplement existing pathophysiological knowledge on important conditions. MRI is an indispensable and non-invasive tool enabling measurement of liver and pancreas, and advances in its automated analysis has made its measurement at scale a reality (9). The availability of MRI scans of liver fat in 32,859 UK Biobank participants has allowed us to understand the genetic contribution to variation in fat content and volume of liver and pancreas (9).

In this study, we aimed to measure the volume and fat content of the liver and pancreas in a large cohort of individuals with type 1 and type 2 diabetes and use the largest available samples with genetic association results to test the causal role of liver and pancreas fat content and volume in the etiology of type 1 and type 2 diabetes using an MR approach.

RESEARCH DESIGN AND METHODS

Data Sources and Study Participants

The UK Biobank Study

We used data from the UK Biobank for the MRI study of liver and pancreas

volume and fat content (10). For the current study, we included 32,859 individuals of White British ancestry who underwent the MRI scan. Type 1 diabetes and type 2 diabetes were defined as binary outcomes from ICD-9 and ICD-10 medical billing codes. The UK Biobank has approval from the North West Multicenter Research Ethics Committee (<https://www.ukbiobank.ac.uk/ethics/>), and these ethics regulations cover the work in this study.

Image Processing

The methods have previously been described in detail (9). In preprocessing, we blended the six separate Dixon neck-to-knee acquisitions, applying bias field correction and automated correction of fat/water swaps. We used the Phase Regularized Estimation using Smoothing and Constrained Optimization (PRESCO) algorithm to estimate proton density fat fraction (PDFF) in the liver and pancreas multiecho slices. To segment organs, we manually annotated organs on the three-dimensional Dixon neck-to-knee acquisition (liver) and T1-weighted three-dimensional pancreas acquisition (pancreas). Annotations were manually inspected to ensure accuracy before use in modeling. We trained a modified U-net convolutional neural network on each modality and applied this to data from all participants. We estimated volumes by counting voxels and multiplying by the size of each model.

Genome-Wide Association Studies of Type 1 and Type 2 Diabetes

Summary-level association results were extracted from the largest publicly available genome-wide association study (GWAS) of type 1 diabetes from the meta-analysis of 9,358 case and 15,705 control subjects (11) and type 2 diabetes from a recent meta-analysis of 55,005 case and 400,308 control subjects of European ancestry (12). The UK Biobank participants were not part of these GWAS. Details on the demographics of the cohorts participating can be found in the respective publications.

FinnGen Study

We used GWAS summary statistics from FinnGen (13) to validate our findings. The GWAS of type 1 diabetes included 2,649 case and 183,674 control sub-

jects, and the GWAS of type 2 diabetes included 29,166 case and 183,185 control subjects. The definitions of disease and population characteristics are summarized in Supplementary Table 1.

Genetic Instrument

We used genetic variants associated with four different exposures, including liver and pancreas fat content and volume. The GWAS has previously been described (9), but in summary, we included participants who self-reported their ancestry as “White British” and who clustered with this group in a principal components analysis. We used BOLT-LMM and included age at imaging visit, age squared, sex, imaging center, scan date, scan time, and genotyping batch as fixed-effects covariates and genetic relatedness as a random effect to control for population structure and relatedness.

For each instrument, we used independent variants associated ($P < 5 \times 10^{-8}$) with each exposure in the UK Biobank. This included 10 variants associated with liver fat (explained 4.6% of the observed variance), 11 variants associated with liver volume (2%), 9 variants associated with pancreas fat (1.9%), and 17 variants associated with pancreas volume (2.3%) (Supplementary Table 2A) (14). The minor allele frequencies of these variants ranged between 0.013 and 0.495.

We extracted estimates of these variants on risk of type 1 (11) and type 2 (12) diabetes (Supplementary Table 2). For genetic variants not present in these GWAS, we selected proxy single nucleotide polymorphisms in linkage disequilibrium ($r^2 > 0.7$) using all European populations from 1000 Genomes phase 3, HapMap, or a reference panel consisting of 379,396 individuals of European ancestry from the UK Biobank (Supplementary Table 2B).

Statistical Analysis

To understand how liver and pancreas fat and volume are associated with risk of type 1 and type 2 diabetes, we performed a logistic regression adjusting for age, sex, height, BMI, imaging center, imaging date, and scan time.

For examination of whether the associations are likely causal, we used inverse-variance weighted (IVW) two-

sample MR as our main analysis (15) to estimate the effect of a 1-SD increase in the four exposures on risk of type 1 and type 2 diabetes. In the absence of horizontal pleiotropy (when the genetic variants are associated with the outcome through pathways other than the exposure) or when horizontal pleiotropy is balanced, the IVW method provides an unbiased effect estimate. In addition, we performed several sensitivity analyses, including weighted median, MR-Egger, mode-based estimate, and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO), to assess and account for potential horizontal pleiotropy. All of the analyses were performed with the Mendelian-Randomization package in R (16). We used the “random” model in IVW and MR-Egger (to allow for the presence of heterogeneity in our instruments) and used the “penalized” parameter to penalize variants with heterogeneous causal estimates. We performed MR-PRESSO using the MR-PRESSO package in R (17). In all of the above analyses, effects were aligned to the exposure-increasing allele reported in previously published work (9).

Data and Resource Availability

Data used in this study can be accessed as follows: type 1 diabetes (<https://www.ebi.ac.uk/gwas/publications/32005708>), type 2 diabetes (<https://diagram-consortium.org>), pancreas and liver fat content/volume (http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90016001-GCST90017000/GCST90016676/).

RESULTS

Characteristics of 32,859 individuals of White British ancestry with MRI scan data from the UK Biobank are presented in Table 1. The mean (SD) age was 63.9 (7.5) years, and 51.5% of participants were women. After adjustment for sex, imaging center, and scan date and time, liver and pancreas volumes were negatively associated with age (−9.9 mL or −0.03 SD/year for liver volume and −0.54 mL or −0.03 SD/year for pancreas volume), pancreas fat was positively associated with age (0.22% or 0.026 SD/year), liver fat was positively associated with age until age 60 years, and from age 60 years onward there was a subtle decline in liver fat (Fig. 1).

Liver Fat

In our observational study with use of data from the UK Biobank, higher liver fat was associated with higher risk of type 2 diabetes (odds ratio [OR] per 1 SD (5.06%) higher liver fat 2.16 [95% CI 2.02, 2.31]); $P = 1e-105$). The two-sample IVW MR provided evidence for a causal role of liver fat in risk of type 2 diabetes with an OR of 1.27 (1.08, 1.49); that is an average 27% increased risk of type 2 diabetes per SD higher liver fat (Table 2 and Fig. 2A). In sensitivity analyses with use of the weighted median (1.29 OR), MR-Egger (1.45 OR), and mode-based method (1.28 OR) there were similar results. There was no evidence of heterogeneity from MR-Egger. Using MR-PRESSO, we found evidence for pleiotropy ($P_{\text{global test}} < 0.001$). Results from MR-PRESSO after outlier correction were slightly stronger

(three outliers removed [those near *APOE*, *GPAM*, and *C2orf16*], OR 1.27 [1.21, 1.34]) (Supplementary Tables 3 and 4). The liver fat-increasing alleles at *GPAM* and *C2orf16* were associated with lower risk of type 2 diabetes ($P = 0.0043$ and $8.3e-5$, respectively) (Supplementary Fig. 1).

Observationally, higher liver fat was associated with lower risk of type 1 diabetes in the UK Biobank (OR 0.79 [95% CI 0.65, 0.96]; $P = 0.018$). We did not find any evidence of causality between liver fat and risk of type 1 diabetes (OR 1.07 [0.90, 1.27] per SD higher liver fat) (Table 2 and Fig. 2B). Results from the three sensitivity methods were similar. There was no evidence of heterogeneity from MR-Egger. Using MR-PRESSO, we found no evidence for pleiotropy ($P_{\text{global test}} = 0.34$) (Supplementary Tables 3 and 4 and Supplementary Fig. 2).

Liver Volume

Observationally, higher liver volume was associated with higher risk of type 2 diabetes (OR 2.11 [95% CI 1.96, 2.27] per 1 SD (1.38 L) higher liver volume; $P = 7e-90$) in the UK Biobank. We did not find any evidence of causality between liver volume and risk of type 2 diabetes (OR 1.37 [0.82, 2.27] per SD higher liver volume) (Table 2 and Fig. 2A). However, results of sensitivity analyses with use of the weighted median (1.42 [1.17, 1.73]) and mode-based method (1.40 [1.11, 1.77]) provided evidence for a causal association. There was no evidence of heterogeneity from MR-Egger. Using MR-PRESSO, we found evidence for pleiotropy ($P_{\text{global test}} < 0.001$). The

Table 1—Study population characteristics, UK Biobank

	Liver fat	Liver volume	Pancreas fat	Pancreas volume
No. of participants	32,858	32,859	25,617	31,758
% female	51.5	51.5	51.2	51.4
Age (years), mean (SD)	63.9 (7.52)	63.9 (7.52)	64.2 (7.48)	63.8 (7.52)
BMI (kg/m ²), mean (SD)	26.5 (4.36)	26.5 (4.37)	26.5 (4.31)	26.5 (4.34)
Height (cm), mean (SD)	169 (9.26)	169 (9.26)	169 (9.26)	169 (9.25)
Type 2 diabetes, <i>n</i> (%)	1,002 (3.53)	1,004 (3.52)	716 (3.33)	968 (3.54)
Type 1 diabetes, <i>n</i> (%)	117 (0.41)	118 (0.41)	73 (0.34)	114 (0.42)
Mean (SD) of the image-derived phenotype in all	5.06% (5%)	1.38 (0.3) L	10.41% (7.9%)	0.06 (0.018) L
Mean (SD) of the image-derived phenotype in females	4.43% (4.7%)	1.28 (0.25) L	8.34% (6.7%)	0.06 (0.016) L
Mean (SD) of the image-derived phenotype in males	5.73% (5.2%)	1.49 (0.3) L	12.6% (8.5%)	0.06 (0.019) L

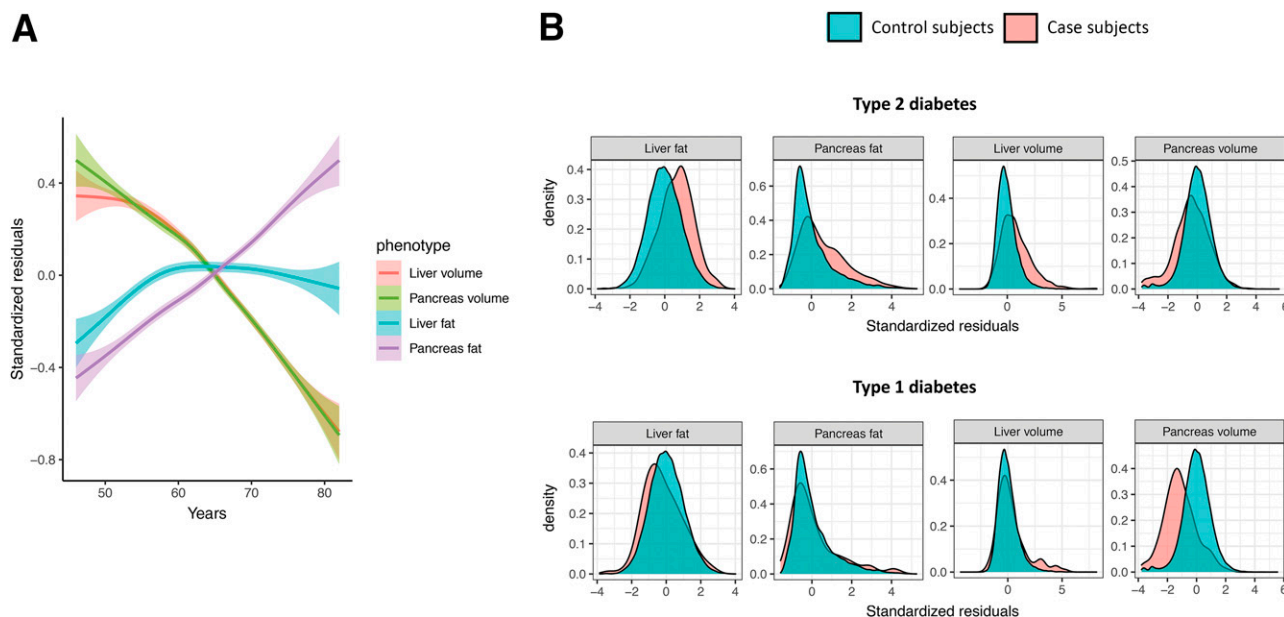


Figure 1—A: Relationship between liver and pancreas fat and volume and age within the UK Biobank. Each trait is standardized, so that the y-axis represents SDs, after adjustment for imaging center and date. The trend is smoothed with use of a generalized additive model with smoothing splines for visualization purposes. B: The density plots of liver and pancreas fat and volume in type 1 and type 2 diabetes case and control subjects within the UK Biobank study.

outlier correction did not alter the inference of the results after removal of six outliers (OR 1.44 [1.24, 1.69]) (Supplementary Tables 3 and 4 and Supplementary Fig. 3). These variants included those near *GSTA2*, *GCKR*, and *PDI3* (where the liver volume-increasing allele was associated with lower risk of type 2 diabetes), and *MAU2*, *RSPO3*, and 16:53812783 (where the liver volume-increasing allele was associated with higher risk of type 2 diabetes).

Observationally, there was no association between liver volume and type 1 diabetes (OR 1.04 [95% CI 1.00, 1.09]; $P = 0.065$) and we did not find any evidence of causality (OR 0.92 [0.67, 1.27] per SD higher liver volume) (Table 2 and Fig. 2B). Comparison of results from the three sensitivity methods indicated no evidence of pleiotropy. There was no evidence of heterogeneity from MR-Egger. Using MR-PRESSO, we found no evidence for pleiotropy ($P_{\text{global test}} = 0.15$) (Supplementary Table 3 and Supplementary Fig. 4).

Pancreas Fat

Observationally, higher pancreatic fat was associated with higher risk of type 2 diabetes (OR 1.42 [95% CI 1.34, 1.51] per SD [10.41%] higher pancreas fat; $P = 3e-31$). However, we did not find

any evidence of causality between pancreas fat and risk of type 2 diabetes (OR 1.02 [0.76, 1.37]) (Table 2 and Fig. 2A). Similar results were obtained with the other three sensitivity methods. There was no evidence for unbalanced horizontal pleiotropy from MR-Egger. Using MR-PRESSO, we found evidence for pleiotropy ($P_{\text{global test}} < 0.001$). Removal of five outliers did not alter the inference of the results (OR 1.06 [0.87, 1.29]) (Supplementary Tables 3 and 4). Among nine variants associated with pancreas fat, fat-increasing alleles at three variants (near *ABO*, *FAM25C*, and rs4733612) were associated with higher risk of type 2 diabetes ($P = 3.9e-7$, 0.036, and $2.6e-5$, respectively), while pancreas fat-increasing alleles near *CEBPB*, *PEPD*, and *PLEKHM3* were associated with lower risk of type 2 diabetes ($P = 5.30e-6$, 0.016, and 0.0019) (Supplementary Fig. 5).

Observationally, there was no association with risk of type 1 diabetes (OR 1.08 [95% CI 0.87, 1.35] per SD higher pancreas fat; $P = 0.49$) and we did not find any genetic evidence of causality (OR 1.26 [0.82, 1.93]) (Table 2 and Fig. 2B). Similar results were obtained with the sensitivity methods. The intercept from the MR-Egger regression test provided evidence for

some unbalanced horizontal pleiotropy. The Q test did not show evidence of heterogeneity in the effect of pancreas fat variants on type 1 diabetes (Q statistic 7.8). Using MR-PRESSO, we found evidence for pleiotropy ($P_{\text{global test}} = 0.005$) (Supplementary Tables 3 and 4). Removing one outlier did not alter the inference of the results (OR 1.47 [1.00, 2.12]).

Pancreas Volume

Observationally, higher pancreatic volume was associated with lower risk of type 2 diabetes (OR 0.73 [95% CI 0.68, 0.78] per SD [0.06 L] higher pancreas volume; $P = 1.31e-23$). Consistently, the two-sample IVW MR provided evidence for a causal role of pancreas volume in risk of type 2 diabetes, with an OR of 0.76 (0.62, 0.94), i.e., an average 24% decreased risk of type 2 diabetes per SD higher pancreas volume (Table 2 and Fig. 2A). Sensitivity analyses with use of the weighted median (0.81) and mode-based method (0.83) provided similar results. MR-Egger yielded an OR of 0.22 (0.11, 0.43). There was some evidence of heterogeneity from MR-Egger. Using MR-PRESSO, we found evidence for pleiotropy ($P_{\text{global test}} < 0.001$). Results from MR-PRESSO after outlier correction were slightly attenuated (three outliers removed, OR 0.83 [0.74, 0.94])

Table 2—Results of the MR study testing causal association between liver and pancreas fat/volume and type 1 and type 2 diabetes

Analysis	OR	Lower CI	Upper CI	<i>P</i>	Egger intercept	Heterogeneity: <i>Q</i> , <i>P</i>	<i>I</i> ² Egger
Liver fat vs. type 2 diabetes							
IVW	1.269	1.079	1.492	0.018		80.4, <i>P</i> < 0.001	
Weighted median	1.288	1.207	1.375	3E−14			
MR-Egger	1.450	1.142	1.842	0.016	−0.020, <i>P</i> = 0.19	64.1, <i>P</i> < 0.001	0.98
MBE	1.283	1.204	1.369	3E−14			
Liver fat vs. type 1 diabetes							
IVW	1.066	0.896	1.268	0.49		11.5, <i>P</i> = 0.24	
Weighted median	1.087	0.891	1.325	0.41			
MR-Egger	1.025	0.772	1.360	0.87	0.006, <i>P</i> = 0.73	11.4, <i>P</i> = 0.18	0.98
MBE	1.057	0.882	1.267	0.55			
Liver volume vs. type 2 diabetes							
IVW	1.366	0.822	2.270	0.26		305.6, <i>P</i> < 0.001	
Weighted median	1.418	1.165	1.725	0.00048			
MR-Egger	0.959	0.320	2.873	0.94	0.028, <i>P</i> = 0.49	289.0, <i>P</i> < 0.001	0.70
MBE	1.400	1.106	1.773	0.0052			
Liver volume vs. type 1 diabetes							
IVW	0.922	0.669	1.270	0.63		15.8, <i>P</i> = 0.11	
Weighted median	0.819	0.583	1.150	0.25			
MR-Egger	0.884	0.435	1.797	0.74	0.003, <i>P</i> = 0.90	15.8, <i>P</i> = 0.07	0.68
MBE	0.826	0.545	1.252	0.37			
Pancreas fat vs. type 2 diabetes							
IVW	1.019	0.757	1.373	0.90		86.2, <i>P</i> < 0.001	
Weighted median	0.956	0.810	1.129	0.60			
MR-Egger	2.227	0.387	12.827	0.40	−0.052, <i>P</i> = 0.40	77.5, <i>P</i> < 0.001	0.35
MBE	0.870	0.681	1.110	0.26			
Pancreas fat vs. type 1 diabetes							
IVW	1.255	0.818	1.925	0.33		22.2, <i>P</i> = 0.005	
Weighted median	1.656	1.120	2.449	0.011			
MR-Egger	0.072	0.015	0.351	0.014	0.191, <i>P</i> = 0.01	7.8, <i>P</i> = 0.35	0.28
MBE	1.895	0.796	4.514	0.15			
Pancreas volume vs. type 2 diabetes							
IVW	0.761	0.620	0.935	0.02		83.1, <i>P</i> < 0.001	
Weighted median	0.805	0.693	0.934	0.004			
MR-Egger	0.220	0.112	0.433	0.00063	0.069, <i>P</i> = 0.002	42.2, <i>P</i> = 0.0001	0.00
MBE	0.825	0.639	1.066	0.14			
Pancreas volume vs. type 1 diabetes							
IVW	1.550	0.845	2.844	0.18		86.8, <i>P</i> < 0.001	
Weighted median	1.159	0.735	1.826	0.53			
MR-Egger	13.425	1.159	155.453	0.057	−0.121, <i>P</i> = 0.10	70.8, <i>P</i> < 0.001	0.35
MBE	0.871	0.429	1.771	0.70			

The ORs are per 1 SD higher liver and pancreas fat/volume. MBE, mode-based estimate.

(Supplementary Tables 3 and 4). The variants excluded were those near *RTL1*, *CTRB2*, and *ABO*.

Observationally, higher pancreas volume was associated more strongly with lower risk of type 1 diabetes (OR 0.42 [95% CI 0.36, 0.48] per SD higher pancreas volume; *P* = 3e−33) in comparison with type 2 diabetes. However, we did not find any evidence of causality between pancreas volume and risk of type 1 diabetes (OR 1.55 [0.85, 2.84]) (Table 2 and Fig. 2B). Similar results were obtained with the sensitivity methods. The MR-Egger regression did not provide strong evidence for unbalanced horizon-

tal pleiotropy. Using MR-PRESSO, we found evidence for pleiotropy ($P_{\text{global test}} < 0.001$). The MR estimates for type 1 diabetes did not alter the inference of the results after removal of four outliers (OR 0.96 [0.67, 1.36]) (Supplementary Tables 3 and 4).

All the MR results were replicated with use of FinnGen data (Supplementary Table 5).

CONCLUSIONS

We provide genetic evidence that higher fat in the liver and lower pancreas volume are both causally associated with higher risk of type 2 diabetes. We did not identify evidence for a causal role

of pancreas fat in type 2 diabetes risk or pancreas volume in type 1 diabetes risk. We used the largest study samples available and performed detailed investigation of possible violations of MR assumptions. We found evidence of pleiotropy for some variants and performed robust sensitivity analyses to test the assumptions of MR and corrected for it if violated.

Liver Fat

The strong genetic evidence we found for a causal role of higher liver fat in risk of type 2 diabetes is consistent with recent MR studies showing a causal

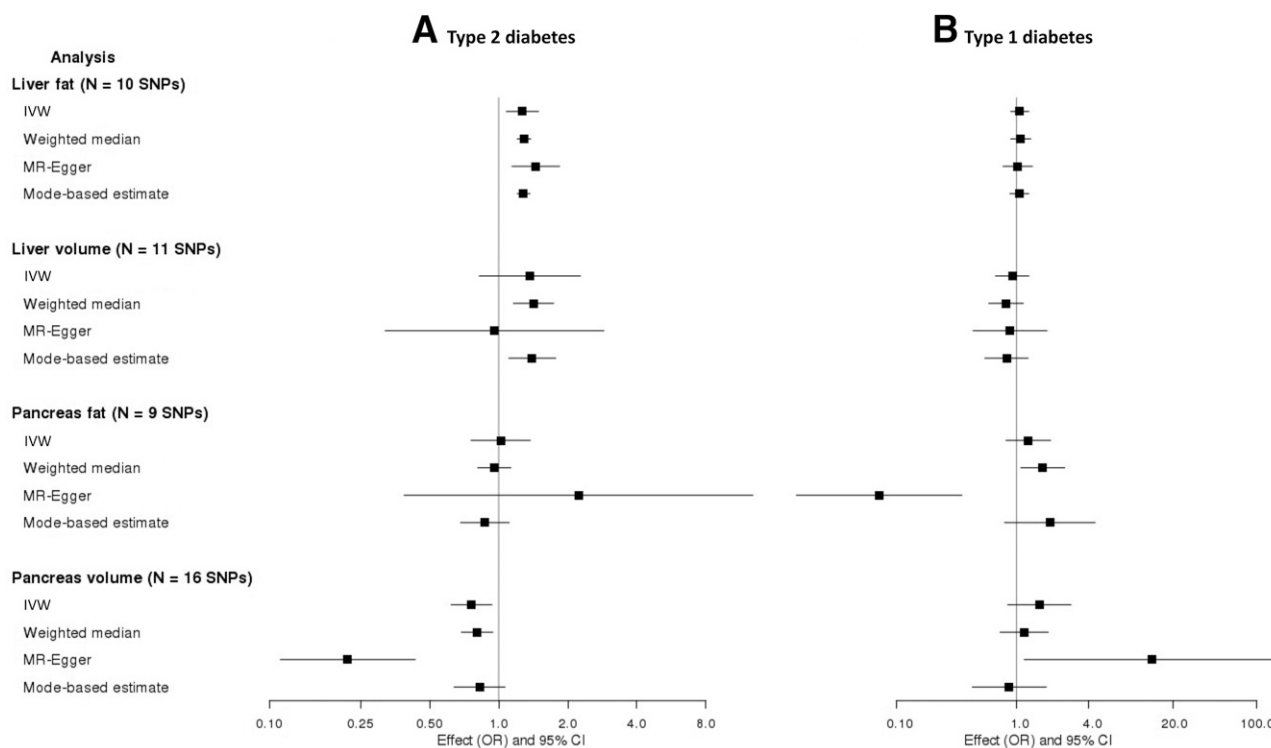


Figure 2—The IVW, weighted median, Egger, and mode-based two-sample MR results for type 2 diabetes (A) and type 1 diabetes (B). Only 16 of the 17 pancreas volume single nucleotide polymorphisms were present in each GWAS. The error bars represent the 95% CIs of the MR estimates in OR per SD change in genetically determined liver and pancreas fat/volume.

association between non-alcoholic fatty liver disease or its markers (ALT and AST) and higher risk of type 2 diabetes (18,19). Using a unique genetic approach, we recently identified 36 genetic variants associated with a favorable adiposity (higher adiposity but a favorable metabolic phenotype and lower risk of type 2 diabetes) and showed that lower liver fat is the key mechanism that protects against risk of type 2 diabetes and other related cardiometabolic diseases in spite of higher adiposity (20). Future work will be needed to expand MR from two-way analyses to a full causal network for type 2 diabetes, as the factors and comorbidities influencing this disease, including risk for liver disease progression, are extraordinarily complex. The link between liver fat and type 1 diabetes as reported in previous studies is less clear with some limited and inconsistent data (21). In our observational analysis, we found a negative association between liver fat and risk of type 1 diabetes in UK Biobank. The explanation could be that *de novo* lipogenesis in the liver falls when insulin production stops in type 1 diabetes;

therefore, liver fat change is a consequence of insulin loss rather than a cause in type 1 diabetes. Consistently, our results provide no evidence for a causal role of higher liver fat in risk of type 1 diabetes.

Liver Volume

Although we did not find any evidence of a causal effect between liver volume and risk of type 2 diabetes in our main MR analysis, our sensitivity MR analyses with correction for bias in our genetic instrument provided evidence for a causal association between greater liver volume and higher risk of type 2 diabetes. The link between greater liver volume and type 2 diabetes could, however, be a reflection of the correlation between greater liver fat and greater liver volume ($r = 0.20$ [95% CI 0.19, 0.21] in our UK Biobank data) as well as the correlation between obesity and liver volume (22).

Pancreas Fat

Observational studies of pancreatic fat provide inconsistent evidence regarding whether pancreatic fat is itself a driver of β -cell dysfunction and type 2

diabetes. Results from the Diabetes Remission Clinical Trial (DiRECT) in the UK demonstrated that the remission of type 2 diabetes was associated with a major reduction in liver triglyceride export and a small, but significant, decrease in pancreatic fat content (23). Conversely, weight regain and return of diabetes were shown to be associated with increased liver and pancreatic fat and re-emergence of β -cell dysfunction (24). The sequence of events suggest that the disease process may be triggered by deposition of ectopic fat in the pancreas, causing β -cell dysfunction and type 2 diabetes (25). However, results of other studies indicate no association between type 2 diabetes and pancreatic fat with use of either computed tomography or histology at autopsy (26). All of these studies are based on small numbers of selected individuals, and our study is the first large-scale one to examine the causal effect of pancreatic fat in diabetes risk. Our results may be consistent with the explanation that higher fat in the pancreas observed in people with type 2 diabetes is secondary to disease or a result of a higher general obesity, but

there are some caveats as discussed below.

At the individual level, variants associated with greater pancreatic fat can be divided into two groups with opposite effects on risk of type 2 diabetes. Studies of these individual variants can provide further insight into the role of pancreatic fat in type 2 diabetes. The allele with the strongest effect on pancreatic fat (in *PEPD*) was associated with lower risk of type 2 diabetes. This allele is also associated with higher body and trunk fat percentage (data from White British in the UK Biobank). The second pancreatic fat-increasing allele (near *CEBPB*) has been shown to be associated with lower risk of type 2 diabetes in a multiethnic analysis (27) but has not been shown to be associated with any other trait/disease. The third pancreatic fat-increasing allele is located in *PLEKHM3* and is not associated with any other trait/disease. However, whether these variants lead to differential location of fat within the pancreas is unknown. Previous studies have shown that fat distribution varies significantly between the head of the pancreas and its other sections (28). This heterogeneity may differentially impact pancreatic function and possibly the development of type 2 diabetes.

Pancreas Volume

Our results provide the first genetic evidence that the decrease in pancreas volume may be causal of type 2 diabetes. Our results support the hypothesis that underlying mechanisms associated with reduced pancreatic volume precede diagnosis of type 2 diabetes. However, we did not see any evidence for a causal role of reduced pancreas volume in the risk of type 1 diabetes, which could be explained by two main factors. First, our genetic instrument for pancreas volume is based on MRI scan data of pancreas in adults with a mean age of 63.8 ± 7.52 years. It is possible that adult pancreas volume does not correlate with pancreas volume in childhood when type 1 diabetes starts. However, observationally, pancreas volume had a stronger association with the risk of type 1 diabetes versus type 2 diabetes in our data. Second, we had less power to detect a causal association with risk of type 1 diabetes compared with type

2 diabetes (9,358 cases vs. 55,005 cases, respectively). However, the direction of effect from the MR study was not consistent with a tentative causal effect between reduced pancreas volume and higher risk of type 1 diabetes. The variant with the strongest effect on pancreas volume is located near *CTRB2* and has opposing effects on risk of type 1 and type 2 diabetes; the pancreas volume-increasing allele is associated with lower risk of type 2 diabetes and higher risk of type 1 diabetes. Given that the pathogenesis of type 1 and type 2 diabetes are clearly different, it could be that the process driving the development of the former, probably autoimmune reaction, may override any effect of organ size. Furthermore, there may be some variants linked to higher volume that may also be linked to greater likelihood of an autoimmune reaction. The minor allele of a correlated variant (rs7202877; $r^2 = 0.66$) was previously identified to be a risk factor for type 1 (29) and a protective factor for type 2 (30) diabetes. The protective effect of the allele against type 2 diabetes has been reported to be associated with glucagon-like peptide 1-stimulated insulin secretion (31). Moreover, a recent interventional study showed that weight gain and prolonged diabetes duration often lead to smaller pancreases, while weight loss reverses this effect, a narrative supportive of the relationship of pancreatic size and type 2 diabetes (32), although our work adds support for pancreatic size being causal for type 2 diabetes, opening the potential for a bidirectional relationship.

Our study had some limitations: 1) We used organ volume and fat content measured in adulthood, which could bias the association with type 1 diabetes toward the null effect. 2) For some genetic variants we used as instruments, the causal genes and therefore the biological mechanisms are unknown, which makes it difficult to test bias and pleiotropy. However, we used rigorous sensitivity tests that supported the main results. 3) Our measurement of pancreas volume does not differentiate between endocrine and exocrine pancreas, and more specific data are needed to understand the role of β -cell mass or exocrine inflammation in mechanisms that link reduced pancreas volume to higher risk of diabetes. 4) The

phenotyping of liver and pancreas volume/fat was performed on tractable measures derived from image segmentation. Although it is possible that some imaging artifacts are introduced in the results, any variance due to this is likely negligible given the size of the cohort and could not have affected our genetic instruments. 5) Using three-point Dixon MRI of the pancreas, we may not have perfectly captured the pathological areas of pancreatic fat in comparison with the more sophisticated technique of the magnetic resonance image “biopsy” method (MR-opsy) (28). However, MR-opsy is not practical for the very large cohorts required for genetic studies, the scale of which demands automated analysis, and the overall impact of the method we used in the current study on the PDFF values and therefore the genetic associations would be minimal. 6) The small difference (~ 1.25 -fold) in pancreas fat content between people with and without type 2 diabetes, and the wide range among people, raises the question of sensitivity of our approach to detect a genuine difference. By taking an MR approach and using a strong instrument for pancreas fat and a large sample size, we had 99% power to detect any association between pancreas fat and risk of type 2 diabetes. However, we suggest that replication of the association between the instrument and pancreas fat in an independent cohort would be valuable. 7) The MRI-derived phenotypes represent the tissue as a whole, without investigation of within-organ heterogeneity, e.g., differences in the regional distribution of fatty deposits within the liver and pancreas or differences in cell type or tissue sections. Also, the present set of parameters does not account for differences in organ shape or position. 8) This study was conducted in a cohort of European ancestry. Even modest differences across populations in contributions of common variation to complex traits necessitate broadening the diversity of populations studied (33). Therefore, our findings are only generalizable to the European population. Finally, change in liver and pancreas fat content or volume could also be affected by pathophysiological mechanisms secondary to type 1 or type 2 diabetes. For example, subclinical

exocrine inflammation of the pancreas associated with insulinitis (34), as well as insulin deficiency and the lack of a trophic effect on pancreas exocrine tissue (3), could contribute to reduced pancreatic volume in type 1 diabetes, while atherosclerosis might cause reduction in pancreas volume in type 2 diabetes (35). Future MR studies investigating the role of type 1 and type 2 diabetes on changes in these features are needed to understand the role of other mechanisms or whether there is a mutual causal effect. We hope other groups can validate or expand our findings in relevant data sets, should they exist with sufficient power.

In summary, our results are in line with a causal role for higher liver fat and reduced pancreas volume in type 2 diabetes etiology and show consistency in sensitivity analyses. Given the worldwide increasing prevalence of type 1 and type 2 diabetes, better understanding of the underlying mechanisms involving liver and pancreas volume and fat content may provide new insights into preventing and treating diabetes.

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takes responsibility for the integrity of the data and the accuracy of the data analysis.

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