Determinants of hepatic insulin clearance – results from a Mendelian Randomization study

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Short running title

Hepatic insulin clearance is not causally associated with elevated liver fat

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Abstract

Aims/Hypothesis: Besides insulin resistance, type 2 diabetes associates with decreased hepatic insulin clearance (HIC). We now tested for causal relationship of HIC to liver fat accumulation or features of the metabolic syndrome.

Methods: HIC was derived from oral glucose tolerance tests with the "Oral C-peptide and Insulin Minimal Models" (n=3311). Liver fat was quantified by magnetic resonance spectroscopy (n=1211). Mendelian Randomization was performed using established single nucleotide polymorphisms (SNPs; 115 for liver fat, 155 alanine-aminotransferase, 37 insulin sensitivity, 37 insulin secretion, 72 fasting insulin, 5285 BMI, 163 visceral fat, 270 waist circumference, 442 triglycerides, 620 HDL-Cholesterol, 193 C-reactive protein, 53 lipodystrophy-like phenotypes).

Results: HIC associated inversely with liver fat ($p<0.003$) and insulin sensitivity ($p<0.0001$). Both liver fat and HIC were independently associated with insulin sensitivity ($p<0.0001$). Neither liver fat nor alanine-aminotransferase were causally linked to HIC, as indicated by Mendelian Randomization ($N_{\text{liver fat}}=1054$, $N_{\text{HIC}}=2254$; $N_{\text{alanine aminotranferase}}=1985$, $N_{\text{HIC}}=2251$). BMI-related SNPs were causally associated with HIC ($N_{\text{BMI}}=2772$, $N_{\text{HIC}}=2259$, $p<0.001$) but not waist circumference-SNPs ($N_{\text{SNPs-waist circumference}}=2751$, $N_{\text{HIC}}=2280$). Genetically determined insulin sensitivity was not causally related to HIC ($N_{\text{insulin sensitivity}}=2752$, $N_{\text{HIC}}=2286$). C-reactive protein and HDL were causally associated with HIC, with higher C-reactive protein and lower HDL leading to higher HIC ($N_{\text{C-reactive protein}}=2660$, $N_{\text{HIC}}=2240$; $N_{\text{HDL}}=2694$, $N_{\text{HIC}}=2275$).

Conclusions: This Mendelian Randomization analysis does not support a causal link between hepatic steatosis and HIC. Other components of the metabolic syndrome seem to compensate peripheral hyperinsulinemia by increasing hepatic insulin extraction.

1. Introduction

Type 2 diabetes is associated with insulin resistance and decreased hepatic insulin clearance (HIC) [1]. Most of the insulin degradation takes place in the liver [2, 3]. The liver seems to have a crucial part in the regulation of peripheral insulin concentrations, as approximately 50-80% of the insulin present in the portal vein is cleared in the first-pass [3, 4].

Nonalcoholic fatty liver disease (NAFLD) includes a group of disorders ranging from a simple steatosis to nonalcoholic steatohepatitis (NASH) [5–7]. NAFLD is characterized by fat accumulation in the liver independent of alcohol consumption. Patients with NAFLD usually present features of the metabolic syndrome such as obesity, insulin resistance, type 2 diabetes and dyslipidemia [5, 7]. An unhealthy lifestyle and a genetic predisposition modulate the progress of the disease [5].

Liver fat content is associated with insulin resistance and reduced HIC, both in people with [8] and without diabetes [9, 10]. However, causality is unclear as patients who suffer from NAFLD are often obese and have marked insulin resistance. Thus, the question whether insulin clearance is associated with insulin resistance independent from liver fat content or whether liver fat accumulation *per se* is the primary cause leading to hepatic and peripheral insulin resistance remains enigmatic. Recent human studies on that matter yielded conflicting results [11, 12]. Furthermore, there is support for the hypothesis that obesity [13], whole-body insulin resistance [14, 15], pulsatile insulin secretion [3, 16] and components of the metabolic syndrome, such as increased visceral fat mass [17, 18], dyslipidemia [17, 18] and subclinical inflammation [19, 20], may exert direct effects on HIC.

We, therefore, evaluated the role of liver fat content and the aforementioned anthropometric and metabolic variables in HIC using Mendelian Randomization (MR). MR is a method for assessing and estimating the causal relationship between an exposure, using genetic instruments with well-known effects on the exposure, and outcome in the presence of confounding factors [21, 22].

2. Material and Methods

2.1 Subjects

3311 individuals of European ancestry, who participated in metabolic screenings within the framework of multiple studies performed at the Department of Medicine IV, University Hospital Tübingen, were retrospectively analyzed. The recruiting criteria include individuals enriched in risk for T2DM (positive family history for diabetes, BMI >27 kg/m^2, women with previously known gestational diabetes or subjects with prediabetes). The study`s participants were considered healthy according to a physical examination and routine laboratory tests. Individuals with diabetes mellitus, pregnant women, history of liver disease and daily consume of more than two alcoholic drinks were excluded from the analyses. All participants provided informed written consent. The Ethics Committee of the Medical Faculty of the University of Tübingen approved the protocol. All studies were performed at the Medical Hospital of the University of Tübingen.

2.2 Magnetic resonance imaging (MRI) and spectroscopy (MRS)

MRI and MRS examinations were performed in the early morning after overnight fasting on a 1.5 T whole-body imager (Magnetom Sonata, Siemens Healthineers, Erlangen, Germany). For quantification of visceral adipose tissue (VAT), a T1-weighted fast spin-echo technique was applied as described [23] and VAT was automatically segmented by a fuzzy-clustering algorithm [24]. Liver fat content was quantified by MRS in a subgroup of 1211 individuals by a single voxel STEAM technique [23]. The diagnostic cut-off for NAFLD was a liver fat content > 5.56% [25] .

2.3 Oral glucose tolerance test and laboratory measurements

All participants underwent a 75 g oral glucose tolerance test (OGTT) (Accu-Check Dextro, Roche) at 8 a.m. following an overnight fast, to diagnose normal glucose tolerance or T2DM according to the current guidelines [26] and to measure insulin and C-peptide. Venous plasma samples were obtained before and 30, 60, 90 and 120 minutes after the glucose ingestion.

Glucose values were measured directly using a bedside glucose analyzer (YSI, Yellow Springs, CO). All other blood samples were immediately put on ice and the serum was centrifuged within 2 hours. Plasma insulin and C-peptide were measured by an immunoassay with the ADVIA Centaur XP Immunoassay System (Siemens Healthineers, Eschborn, Germany). The typical day-to-day variation of the assays (coefficient of variation) is < 5% for insulin and < 6% for C-peptide. Triglycerides (TG), high-density lipoprotein (HDL)-cholesterol levels and alanine aminotransferase (ALT) were measured using the ADVIA XPT clinical chemical analyzer (Siemens Healthineers, Eschborn, Germany). C-reactive protein (CRP) levels were measured using ADVIA XPT (Siemens Healthineers, Eschborn, Germany). HbA1c measurements were performed using the Tosoh glycohemoglobin analyzer HLC-723G8 (Tosoh Bioscience Tokyo Japan).

2.4 Hyperinsulinemic euglycemic clamp

In a subgroup of 534 subjects, a hyperinsulinemic euglycemic clamp was performed as described previously [27]. In short, insulin was infused at a rate of 1.0 mU kg−1 min−1 over 2 h and blood glucose was kept stable by glucose infusion, as needed.

2.5 Genetic instruments

We selected SNPs that are related to liver fat content and ALT, fasting insulin, waist circumference, HDL-cholesterol, TG and CRP levels from the GWAS catalog (https://www.ebi.ac.uk/gwas/search) as well as SNPs related to insulin resistance phenotypes (lipodystrophy-like phenotypes) from the study of Lotta et al. [28]. SNPs related to insulin sensitivity and insulin secretion were selected from the study of Mahajan et al. [29] and SNPs related to VAT from the study of Karlsson et al. [30]. SNPs related to body mass index (BMI) were chosen from the study of Yengo et al. [31]. After excluding rare variants (MAF < 0.005) and pruning variants in high linkage (LD > 0.8), we obtained 115 liver fat SNPs, 155 ALT-SNPs, 37 insulin sensitivity SNPs, 37 insulin secretion SNPs, 72 fasting insulin, 5285 BMI-

SNPs, 163 visceral fat, 270 waist circumference, 442 TG-SNPs, 620 HDL-SNPs, 193 CRP-SNPs, and 53 SNPs lipodystrophy-like phenotypes. All genotyping was performed using a 700-K Infinium Global Screening Array from Illumina (SanDiego, CA, USA). In all analyses, we adjusted for potential population stratification by adjusting for the first 3 principal components of the genotype matrix. We report the median N of the participants in the linear regressions underlying the MR models.

2.6 Calculations

The insulin sensitivity index (ISI), as proposed by Matsuda and de Fronzo [32], was used to estimate insulin sensitivity. HIC was calculated by "Oral C-peptide and Insulin Minimal Models" as described previously [33]. Whole-body insulin clearance was estimated by the ratio of C-peptide and insulin during an euglycemic hyperinsulinemic clamp [34]. The area under the curve (AUC) for insulin clearance was calculated with the trapezoid method.

2.7 Statistical analysis

All statistical analyses were conducted in R 3.5.2 (R Core Team, 2018-12-20). Data are presented as median and interquartile range. Outcome variables in linear regression models were log-transformed to approximate normal distributions. Age, sex and BMI were included as covariant in all regression models. A p-value < 0.05 was considered statistically significant.

We performed MR analyses using the package Mendelian Randomization (Version 0.4.2, 2020-02-24) to test causal determination of HIC by liver fat content and potentially related traits using established SNPs. Four different methods of one-sample MR were used in order to assess and adjust for pleiotropy and heterogeneity and to exclude outliers. The inverse-variance weighted (IVW) method presumes that all genetic variants are valid instrumental variants [35]. The weighted median method assumes that most of the variants are valid [36]. The MR-Egger regression method was chosen to account for potential horizontal pleiotropy

[35], which arises if the variants associated with the intermediary trait are also causally linked the outcome trait. Finally, the Mendelian Randomization Pleiotropy Residual Sum and Outlier (MR-PRESSO) model was used to identify and correct for horizontal pleiotropic outliers [37]. We considered the data as sufficiently supporting a causal link when at least three methods provided significant results.

Moreover, in order to investigate whether the effect of parameters related to metabolic syndrome, such CRP, VAT, waist circumference, HDL and TG on HIC occurs independent from insulin resistance, we adjusted the aforementioned parameters for insulin sensitivity in the Mendelian Randomization models. Given an adjusted effect size of β_{OLS}=-0.11 in the regression of liver fat on HIC and an R^2=0.13 by all deployed genetic variants explaining liver fat, our main two-stage MR model was adequately powered (sensitivity 0.8) to detect an underlying causal effect estimate of β=-0.195 [38].

3. Results

3.1 Subjects characteristics

A total of 63% of the participants were women (n=2097) and 37% men (n=1214), with a median age of 41 years. They had a median BMI of 28 kg/m^2. All demographic and metabolic characteristics are summarized in Table 1. In a subgroup of 1211 subjects with liver fat measurements, the median liver fat content was 3.4%. Of those, 35% (n=429) had NAFLD. A total of 534 participants underwent a hyperinsulinemic euglycemic clamp, of them 54% were women (n=287) and the median age and BMI were 47 (38-55) years and 28 (25-32) kg/m^2, respectively. In the clamp subgroup, liver fat content was measured in 374 subjects. NAFLD was diagnosed in 29% of the participants (n=107).

3.2 Associations

HIC associated positively with insulin sensitivity (β=0.6±0.03, p<0.0001) and inversely with liver fat content (β=-0.1±0.03, p<0.003) (Figure 1 A-B). There was an inverse association between liver fat content and insulin sensitivity (β=-0.7±0.04, p<0.0001) (Figure 1C). Both, liver fat content and HIC were independently associated with insulin sensitivity (β=-0.2±0.03 and β=0.1±0.02 respectively, both p<0.0001). HIC was not associated with the use of oral contraceptives in women (329 women, β=-0.11, p=0.09) or thyroid hormone substitution (586 participants, β=0.06, p=0.17). Testosterone levels were not associated with HIC (N=618, p=0.57).

3.3 Mendelian Randomization

To assess whether there is a causal relation between liver fat content and HIC, we used genetic variants that are associated with liver steatosis as instruments and performed MR analysis using these instruments. Although the MR models indicate that genetically predicted NAFLD causally affected whole-body insulin clearance according to the MR models (β=-0.3±0.08 in IVW model and MR-PRESSO and β=-0.4±0.1 in MR-Egger and weighted

median analysis, p<0.0001 in all MR models; Figure 2A), there was no statistical evidence for a causal impact on HIC (p=0.6; MR-Egger; Figure 2B).

Further, we also tested genetic variants associated with serum ALT level, which is commonly associated with hepatitis/steatohepatitis. Genetically increased ALT did not modulate HIC (p=0.3; MR-Egger) in any of the tested four MR models (Figure 2C).

As MR did not reveal statistical evidence for a causal role of liver fat in HIC, we next tested whether alterations in insulin secretion and sensitivity modify clearance. Genetically determined insulin secretion was not associated with HIC (p=0.8 in MR-Egger). The analysis did not detect a causal relation between insulin sensitivity and HIC (p=0.1in MR-Egger) (Figure 2D). On the other hand, genetically determined fasting hyperinsulinemia, as defined by fasting insulin levels, was detected to causally alter HIC (β=-0.2±0.1, p=0.005 in IVW model; β=-0.3±0.1, p=0.02 in MR-Egger; β=-0.2±0.08, p=0.001 in MR-PRESSO and β=-0.4±0.1, p=0.006 in weighted median method; Figure 2E).

Clinically, features of the metabolic syndrome are most closely related to insulin resistance and hyperinsulinemia. Therefore, we also tested the features of the metabolic syndrome and their association with HIC. First, we performed MR-analysis with genetic instruments of BMI. They indicated that increased BMI causally affected HIC (β=-0.2±0.01, p<0.001 in MR-Egger, IVW and weighted median method). However, waist circumference and VAT, being key components of the metabolic syndrome, were not linked to HIC (p=0.1and p=0.2, respectively in MR-Egger). Moreover, genetic variants of insulin resistance independent from adiposity (genotypes associated with a lipodystrophy pattern, i.e. higher fasting insulin adjusted for BMI, lower HDL cholesterol and higher triglycerides [28]) were not associated with HIC (p=0.1 in MR-Egger; Figure 2F).

We tried to understand, why the metabolic syndrome and phenotypes that cause insulin resistance did not correlate with HIC, whereas MR indicated that BMI causally impacts HIC. Therefore, we investigated separately aspects of the metabolic syndrome and their influence

on HIC. As T2D, insulin resistance and NAFLD are related to the metabolic syndrome, we assumed that other traits such as TG, HDL-cholesterol and systemic inflammation may explain the alterations in HIC in the context of NAFLD. We performed MR-analyses using genetic variants associated with TG levels. These instruments did not show evidence for causality (p=0.8 in MR-Egger), even after adjusting the intermediary trait for insulin sensitivity (p=0.5 in MR-Egger). However, the analysis indicated a causal association of HDL with HIC (β=-0.05±0.02 p=0.03 in IVW; β=-0.1±0.03, p=0.01 in MR-Egger; β=-0.1±0.04, p=0.007 in weighted median method; β=-0.05±0.02, p=0.02 in MR-PRESSO; Figure 2G). This statistical association became stronger after adjusting HDL for insulin sensitivity, suggesting that higher HDL levels are lowering HIC (β=-0.1±0.02, p≤0.001 in the MR regression models). As systemic inflammation is often present in obesity, insulin resistance and metabolic syndrome, we further investigated serum CRP as an instrument. Elevated CRP was related to higher HIC (β=0.1±0.05, p=0.03 in IVW; β=-0.1±0.08, p=0.03 in weighted median method and β=0.1±0.05, p=0.02 in MR-PRESSO) but not in MR-Egger (p=0.1) (Figure 2H). As three of the four methods yielded significant results, we considered the effect as evidence for causal relationship.

After adjusting CRP for insulin sensitivity, genetically elevated CRP showed evidence for a causal connection to higher HIC (β=0.1±0.04, p=0.002 in IVW, β=0.1±0.06, p=0.01 in MR-Egger, β=0.1±0.07, p=0.009 in weighted median method; β=0.1±0.04, p=0.001 in MR-PRESSO). All results of the Mendelian Randomization analysis are detailed in Table 2-4.

4. Discussion

As hepatic insulin clearance regulates how much of the insulin secreted into the portal vein is eliminated in the first pass through the liver, it is a key mechanism in the control of insulin concentrations in the systemic circulation. Lower insulin clearance has been associated with insulin resistance and elevated liver fat content in most [12, 39–41], but not all [11] previous studies. However, it remained an open question, whether hepatic steatosis causally contributes to a lower hepatic insulin extraction and subsequently to systemic hyperinsulinemia and insulin resistance.

Our current results do not support a causal association between liver fat content and HIC. While a statistically non-significant result can never fully exclude causality that was not detectible by our approach, the findings argue against a strong determination of HIC by liver fat. Further on, MR analysis did not support a causal relationship between elevated serum ALT and lower HIC, which also argues against a major impact of steatohepatitis in the determination of HIC. In contrast to hepatic insulin clearance, we detected evidence for liver fat content being causally linked to whole-body insulin clearance, as determined from hyperinsulinemic-euglycemic clamps. An explanation for this differential effect of liver fat on whole-body vs. hepatic insulin clearance could be that liver fat influences peripheral (whole-body) insulin sensitivity [14, 15, 42], which is covered by whole-body clearance, whereas hepatic insulin clearance is a separate physiological process not directly modulated by liver fat.

If liver fat and steatohepatitis appear not to be regulators of HIC, the question arises as to which factors determine it. Based on previous data about the pulsatility of insulin secretion affecting hepatic insulin extraction [3, 16], we investigated the link between insulin secretion and HIC. Of note, reduced insulin pulse amplitudes and loss of pulsatility are associated with lower insulin secretion [43, 44]. However, we also could not detect evidence for a causal association between genetically determined insulin secretion and HIC.

As hyperinsulinemia and insulin resistance are hallmarks of type 2 diabetes and both associate with reduced insulin clearance, we further tested whether systemic insulin sensitivity/resistance may play a role in determining HIC. Though, there was also no evidence for genetically predicted insulin sensitivity being causally associated with HIC. It should be mentioned that insulin secretion and insulin sensitivity are inherently difficult to model in this context. Insulin is not measured in the portal, but rather in peripheral blood, thus after HIC has already taken place. Insulin resistance can also be confounded by obesity and increased VAT that potentially exert direct effects on HIC. Therefore, in an attempt to model insulin resistance as a pathophysiologic concept, we chose to test variants related to metabolic syndrome characterized by lipodystrophy-like fat distribution, hypertriglyceridemia and low HDL-cholesterol levels [45]. Even using these variants as genetic instrument for insulin sensitivity we did not detect a significant causal influence of insulin resistance on HIC. Genetically determined abdominal obesity, as defined by high waist circumference or elevated VAT, which is also associated with insulin resistance, showed no evidence of a causal connection with HIC.

As a next step, we investigated additional phenotypes, that are strongly associated with insulin resistance, such as low HDL-cholesterol and high TG levels and systemic inflammation, all modeled using genetic variants and tested for a causal link with HIC. Genetically determined TG did not demonstrate evidence for a causal role in HIC, but HDL-cholesterol and CRP showed evidence for a causal association with HIC. However, the effect direction of influence was opposite to what had been expected from simple associations. Genetically low HDL and genetically elevated C-reactive protein levels both seem to increase HIC, using underlying linear regression models adjusted for genetic stratification and insulin sensitivity.

Consequently, low HDL-cholesterol and systemic inflammation could be acting against hyperinsulinemia by increasing HIC.

The role of systemic inflammation and interleukin 6 (IL-6) levels on insulin clearance has already been investigated at the mechanistic level. IL-6 increases insulin clearance by raising the expression of insulin degrading enzyme [20] (IDE), which is known as one key mechanism of insulin clearance in the liver [46]. Decreased levels of the encoded enzyme are associated with the development of type 2 diabetes [47]. Kurauti et al. demonstrated that IL6-knockout mice had impaired insulin clearance probably due to downregulation of insulin degrading enzyme [20]. In addition to IDE, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1)-mediated insulin uptake is a further important mechanism of HIC [48]. It has been shown that deactivation of *Ceacam1* leads to impaired HIC and consequently to systemic hyperinsulinemia, insulin resistance and hepatic steatosis [48–50]. There could also be a link between CEACAM1 and systemic inflammation, as induction of hepatic CEACAM1 by exenatide in mice not only alleviates insulin resistance and liver steatosis, but also increases the production of proinflammatory markers, such as IL-6 and TNFa [51]. In sum, CRP that is downstream of IL-6 could be a readout of an important regulatory pathway for both IDE and CEACAM1. The causal link in the MR analysis between CRP levels and HIC argue for a relevant contribution of this pathway in humans.

Our study has several limitations. First, gold-standard measurement of hepatic insulin clearance would require portal venous sampling, which is obviously not feasible in human studies. Therefore, we estimated HIC levels from C-peptide and insulin modeling. Also, there are no published genetic variants that are associated directly with HIC, such that we were unable to investigate the downstream effects of the modulation of HIC. This has also precluded bidirectional MR-analyses. Furthermore, horizontal pleiotropy, one of the main possible sources of bias in MR-analyses, could be especially problematic using insulin-related traits, since insulin levels are already influenced by insulin clearance. The establishment of new genetic instruments that associate with insulin secretion and sensitivity based on C-peptide rather than systemic insulin measurements, as well as GWAS for insulin clearance

will be needed to address if HIC causally influences some other traits such as obesity, VAT and peripheral fat. A further limitation is that we didn't perform correction for multiple testing over all the hypotheses tested in our work.

In conclusion, we examined a possible causative link of HIC and liver fat using Mendelian Randomization. Our study argues against a causal connection between hepatic steatosis and HIC, but suggests an influence of hepatic steatosis on whole-body clearance. Phenotypes related to insulin resistance such as lipodystrophy-like phenotypes and visceral obesity did not clearly modulate HIC. In contrast, systemic inflammation and HDL-cholesterol seems to contribute to HIC, which is in agreement with data from animal models.

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Author contribution

A.L. and R.W. analyzed the data and wrote the manuscript. C.W., J.M., F.S., S.S.E., C.D.M., R.V., N.S., A.L.B., A.P. contributed to the interpretation of the data. R.W., A.F., M.H and H-U.H. contributed to the study design, interpretation of the data and reviewed the manuscript.

Conflict of interest

The authors have no conflicts of interest that are directly relevant to the contents of this study.

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TABLE 1:

Demographic characteristics of TUEF-participants

Characteristics	**TUEF-Participants (N=3311)**
Age (years)	41 (30-53; N=3307)
BMI (kg/m²)	28.3 (24-34; N=3299)
Sex (f/m)	63%/37% (N=3311)
HbA1c (%)	5.5 (5.2-5.8; N=3141)
Liver fat content (%)	3.4 (1.4-8.4; N=1211)
≥ 5.56	35% (N=429)
< 5.56	65% (N=782)
fasting insulin (pmol/l)	48 (29-78; N=3272)
hepatic insulin clearance	0.2 (0.1-0.3; N=2640)
inulin sensitivity index (AU)	14.1 (8.5-22.5; N=3226)
insulin secretion (AU)	102 (63-165.5; N=3254)
ALT (IU/l)	24 (17-33; N=2295)
HDL (mg/dl)	52 (43-62; N=3139)
Triglycerides (mg/dl)	100 (71-142; N=3159)
waist circumference (cm)	94 (83-108; N=3244)
VAT (l)	3.1 (1.7-5.0; N=1230)

All values given as median with interquartile range

TABLE 2:

Mendelian Randomization Models showing the effect of the genetic exposures (liver and ALT) for insulin clearance after adjustment for population stratification. An MR-Egger intercept significantly different from 0 indicates directional pleiotropy (the tested variants directly affect the outcome), for which the MR-Egger regression adjusts.

Exposure/Outcome	Methods	Beta	SE	95% CI	p-value
Liver Fat/HIC	IVW	0.008	0.009	-0.01	0.3
(n=1054/2254)	MR-Egger	0.006	0.01	-0.019	0.6
	Weighted median	0.01	0.01	-0.016	0.4
	MR-PRESSO	0.007	0.008	NA	0.3
	MR-Egger intercept	*0.001*	*0.006*	*-0.01*	*0.8*
Liver Fat/Whole-Body Insulin Clearance (clamp-derived)	IVW	-0.3	0.08	-0.497	< 0.001
(n=259/427)	MR-Egger	-0.4	0.1	-0.658	< 0.001
	Weighted median	-0.4	0.1	-0.741	< 0.001
	MR-PRESSO	-0.3	0.08	NA	< 0.001
	MR-Egger intercept	*0.01*	*0.01*	*-0.014*	*0.3*
ALT/HIC	IVW	-0.01	0.07	-0.259	0.1
(n=1985/2251)	MR-Egger	-0.08	0.1	-0.293	0.3

	Weighted median	-0.03	0.1	-0.2	0.7
	MR-PRESSO	-0.1	0.07	NA	0.1
	MR-Egger intercept	*-0.002*	*0.005*	*-0.011*	*0.7*

TABLE 3:

Mendelian Randomization Models showing the effect of the genetic exposures (insulin secretion, insulin sensitivity and fasting insulin) for hepatic insulin clearance after adjustment for principal components. The MR-Egger intercept tests for directional pleiotropy.

Exposure/Outcome	**Methods**	**Beta**	**SE**	**95% CI**	**p-value**
Insulin Secretion/HIC	IVW	0.006	0.08	-0.16	0.9
(n=2739/2289)	MR-Egger	-0.01	0.1	-0.3	0.8
	Weighted median	0.1	0.1	-0.09	0.2
	MR-PRESSO	0.005	0.08	NA	0.9
	MR-Egger intercept	*0.002*	*0.009*	*-0.01*	*0.8*
Insulin Sensitivity/HIC	IVW	0.2	0.1	-0.09	0.1
(n=2752/2286)	MR-Egger	0.2	0.2	-0.138	0.1
	Weighted median	0.09	0.2	-0.3	0.6
	MR-PRESSO	0.2	0.1	NA	0.1
	MR-Egger intercept	*0.003*	*0.007*	*-0.01*	*0.6*
Fasting Insulin/HIC	IVW	-0.2	0.1	-0.4	0.005
(n=2787/2284)	MR-Egger	-0.3	0.1	-0.649	0.02
	Weighted median	-0.4	0.1	- 0.75	0.006
	MR-PRESSO	-0.2	0.08	NA	0.001
	MR-Egger intercept	*0.008*	*0.006*	*-0.005*	*0.2*

TABLE 4:

Mendelian Randomization models showing the effect of the genetic exposures (lipodystrophy-like phenotypes, BMI, waist circumference, VAT, TG, HDL and CRP) for hepatic insulin clearance after adjustment of principal components. The MR-Egger intercept tests for directional pleiotropy.

Exposure/Outcome	Methods	Beta	SE	95% CI	p-value
Lipodystrophy-like phenotypes/HIC	IVW	0.2	0.1	-0.06	0.1
(n=2743/2278)	MR-Egger	0.3	0.2	-0.148	0.1
	Weighted median	0.4	0.2	-0.03	0.07
	MR-PRESSO	0.2	0.1	NA	0.09
	MR-Egger intercept	*-0.003*	*0.007*	*-0.018*	*0.6*
BMI/HIC	IVW	-0.2	0.01	-0.234	< 0.001
(n=2772/2259)	MR-Egger	-0.2	0.02	-0.288	< 0.001
	Weighted median	-0.2	0.02	-0.284	< 0.001
	MR-PRESSO				
	MR-Egger intercept	*0.002*	*0.001*	*0.000*	*0.01*
Waist Circumference/HIC	IVW	-0.1	0.05	-0.222	0.06
(n=2751/2280)	MR-Egger	-0.1	0.08	-0.279	0.1
	Weighted median	-0.2	0.09	-0.389	0.03
	MR-PRESSO	-0.1	0.05	NA	0.05

	MR-Egger intercept	*0.001*	*0.003*	*-0.006*	*0.9*
Waist Circumference */HIC	IVW	0.1	0.07	0.052	0.007
(n=2698/2252)	MR-Egger	0.1	0.1	-0.12	0.3
	Weighted median	0.1	0.1	-0.095	0.2
	MR-PRESSO	0.1	0.07	NA	0.006
	MR-Egger intercept	*0.003*	*0.003*	*-0.003*	*0.3*
VAT/HIC	IVW	0.07	0.05	-0.038	0.2
(n=1068/2249)	MR-Egger	0.1	0.08	-0.063	0.2
	Weighted median	0.03	0.08	-0.133	0.6
	MR-PRESSO	0.07	0.05	NA	0.1
	MR-Egger intercept	*-0.002*	*0.004*	*-0.01*	*0.5*
VAT */HIC	IVW	0.1	0.05	0.019	0.02
(n=1064/2222)	MR-Egger	0.1	0.09	-0.03	0.1
	Weighted median	0.1	0.08	-0.02	0.08
	MR-PRESSO	0.1	0.05	NA	0.01
	MR-Egger intercept	*-0.001*	*0.004*	*-0.009*	*0.7*
TG/HIC	IVW	0.006	0.02	-0.037	0.7
(n=2722/2283)	MR-Egger	-0.005	0.03	-0.064	0.8
	Weighted median	0.02	0.03	-0.039	0.4
	MR-PRESSO	0.006	0.02	NA	0.7
	MR-Egger intercept	*0.001*	*0.003*	*-0.004*	*0.6*
TG */HIC	IVW	0.03	0.02	-0.005	0.08
(n=2673/2255)	MR-Egger	-0.01	0.02	-0.068	0.5
	Weighted median	0.03	0.02	-0.019	0.1
	MR-PRESSO	0.03	0.01	NA	0.08
	MR-Egger intercept	*0.006*	*0.002*	*0.002*	*0.004*
HDL/HIC	IVW	-0.05	0.02	-0.106	0.03
(n=2694/2275)	MR-Egger	-0.126	0.03	-0.197	0.001

	Weighted median	-0.1	0.04	-0.195	0.007
	MR-PRESSO	-0.05	0.02	NA	0.02
	MR-Egger intercept	*0.006*	*0.002*	*0.002*	*0.006*
HDL */HIC	IVW	-0.1	0.02	-0.161	< 0.001
(n=2646/2248)	MR-Egger	-0.1	0.03	-0.226	< 0.001
	Weighted median	-0.1	0.03	-0.209	0.001
	MR-PRESSO	-0.1	0.02	NA	< 0.001
	MR-Egger intercept	*0.004*	*0.002*	*<0.001*	*0.04*
CRP/HIC	IVW	0.1	0.05	0.01	0.03
(n=2660/2240)	MR-Egger	0.1	0.07	-0.03	0.1
	Weighted median	0.1	0.08	0.016	0.03
	MR-PRESSO	0.1	0.05	NA	0.02
	MR-Egger intercept	*0.001*	*0.004*	*-0.007*	*0.9*
CRP */HIC	IVW	0.1	0.04	0.054	0.002
(n=2616/2214)	MR-Egger	0.1	0.06	0.039	0.01
	Weighted median	0.1	0.07	0.049	0.009
	MR-PRESSO	0.1	0.04	NA	0.001
	MR-Egger intercept	*-0.002*	*0.003*	*-0.008*	*0.6*

*adjusted for insulin sensitivity

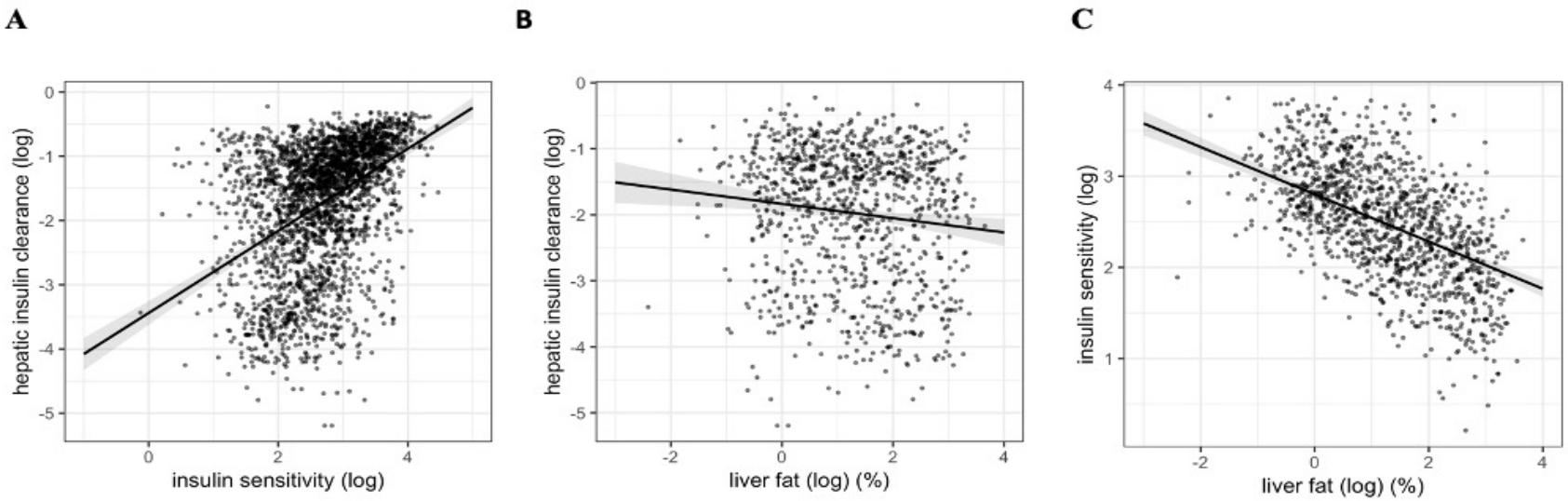
HIC: hepatic insulin clearance, VAT: visceral fat, TG: triglycerides, CRP: C-reactive protein

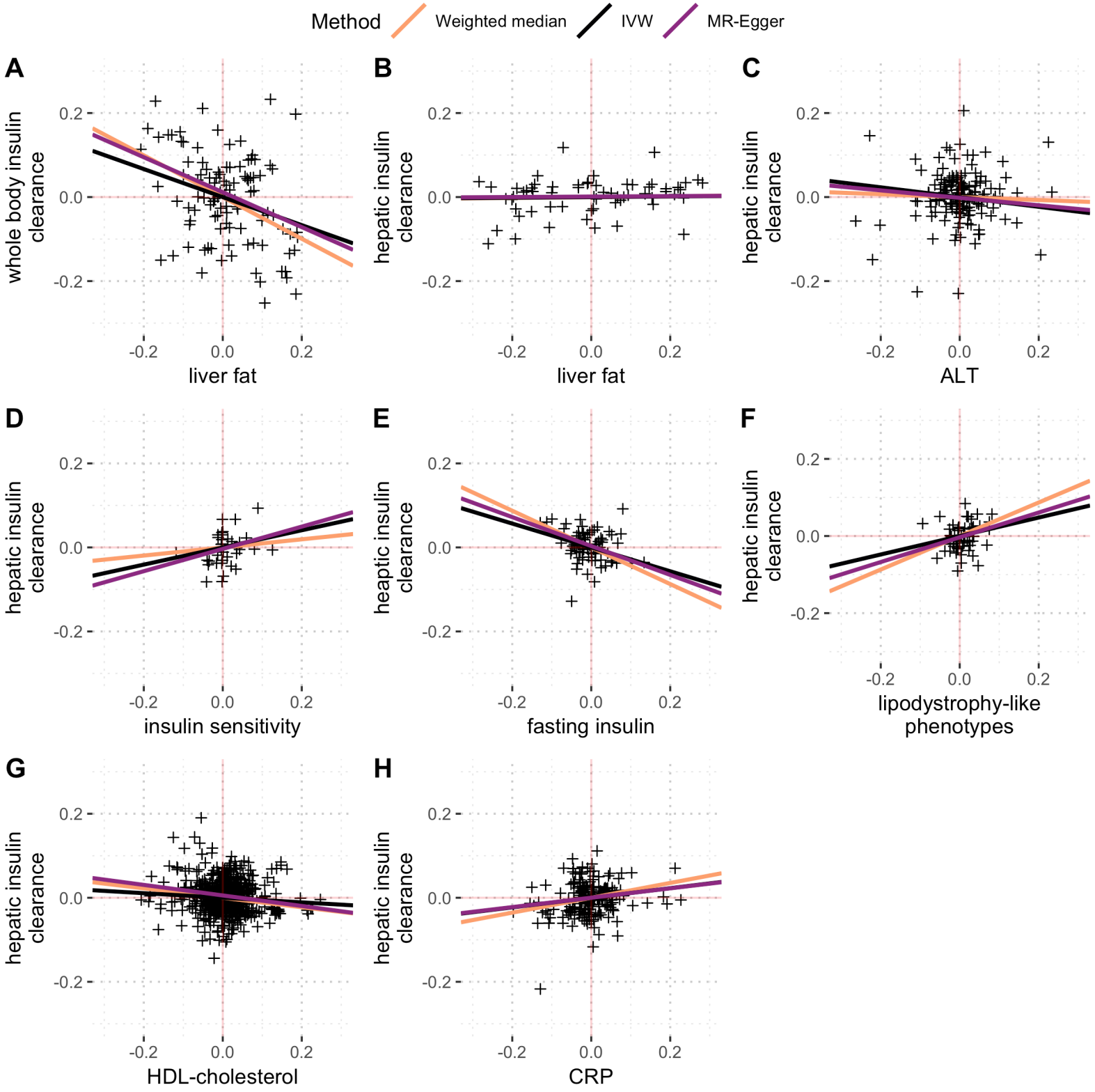
FIGURE 1:

Regression models showing A: association of hepatic insulin clearance with insulin sensitivity, B: inverse association of hepatic insulin clearance with liver fat, C: inverse association of insulin sensitivity with liver fat. All models are adjusted for BMI, age and sex.

FIGURE 2:

MR-plots showing the beta-values (effect sizes) of each used genetic variant (SNPs) on the instrumental variable (x-axis, A and B: liver fat; C: ALT, D: insulin sensitivity; E: fasting insulin; F: lipodystrophy-like phenotypes; G: HDL-cholesterol; H: CRP) and the outcome (y-axis, A: whole-body clearance; B-H: hepatic insulin clearance).


A
hepatic insulin clearance (log)
insulin sensitivity (log)
B
hepatic insulin clearance (log)
liver fat (log) (%)
C
insulin sensitivity (log)
liver fat (log) (%)


Method
Weighted median
IVW
MR-Egger
A
whole body insulin clearance
liver fat
B
hepatic insulin clearance
liver fat
C
hepatic insulin clearance
ALT
D
hepatic insulin clearance
insulin sensitivity
E
heaptic insulin clearance
fasting insulin
F
hepatic insulin clearance
lipodystrophy-like phenotypes
G
hepatic insulin clearance
HDL-cholesterol
H
hepatic insulin clearance
CRP
0.2
0.0
-0.2