The effect of liver enzymes on adiposity: a Mendelian randomization study

Jun Xi Liu[1], Shiu Lun Au Yeung[1], Man Ki Kwok[1], June Yue Yan Leung[1], Shi Lin Lin[1], Lai Ling Hui[1,2], Gabriel Matthew Leung[1], C. Mary Schooling[1,3]

[1]School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

[2]Department of Paediatrics, Faculty of Medicine, the Chinese University of Hong Kong, Hong Kong SAR, China

[3]City University of New York Graduate School of Public Health and Health Policy, New York, NY, USA

Corresponding author:

Dr. C Mary Schooling

School of Public Health

Li Ka Shing Faculty of Medicine

The University of Hong Kong

1/F, Patrick Manson Building (North Wing), 7 Sassoon Road,

Hong Kong SAR, China

Telephone: (852) 3917 6732

Fax: (852) 3520 1945

E-mail: cms1@hku.hk

Conflict of interest: none declared.

Abstract:

Poorer liver function is positively associated with diabetes in Mendelian randomization (MR) studies. Observationally, adiposity is associated with poorer liver function. To clarify the etiology, we assessed the association of liver function with adiposity observationally and using two sample MR for validation.

In the "Children of 1997" birth cohort, we used multivariable linear regression to assess the associations of ALT and alkaline phosphatase (ALP) (IU/L) at ~17.5 years with body mass index (BMI) (kg/m^2). Using MR, genetic variants predicting ALT, ALP and gamma glutamyltransferase (GGT) (100% change in concentration), were applied to genome-wide association studies of BMI, waist circumference (WC) and waist-hip ratio (WHR) (standard deviations) to obtain unconfounded inverse-variance weighting estimates.

Observationally, ALT was positively associated with BMI (0.10, 95% confidence interval (CI) 0.09 to 0.11). ALP was inversely associated with BMI (-0.018, 95% CI -0.024 to -0.012). Using MR, ALT was inversely associated with BMI (-0.14, 95% CI -0.20 to -0.07), but not WC or WHR. ALP and GGT were unrelated to adiposity.

Poorer liver function might not cause adiposity; instead higher ALT might reduce BMI. Whether ALT contributes to diabetes by reducing muscle mass, given the no association of ALT with WC or WHR, requires investigation.

Keywords:

Epidemiology, Mendelian randomization, liver enzymes, adiposity

Abbreviations:

ALT: alanine transaminase; ALP: alkaline phosphatase; GGT: gamma glutamyltransferase; NAFLD: nonalcoholic fatty liver disease; BMI: body mass index; WC: waist circumference; WHR: waist-hip ratio; T2DM: type 2 diabetes mellitus; SEP: socioeconomic position; MR: Mendelian randomizatio; SNP: single nucleotide polymorphisms; GWAS: genome-wide association study; GIANT:

Genetic Investigation of ANthropometric Traits; GIANTUKB: 2018 GIANT and UK Biobank meta-analysis; SD: standard deviation; IVW: inverse variance weighting; WM: weighted median.

Introduction:

Observationally, poorer liver function, particularly nonalcoholic fatty liver disease (NAFLD), is associated with higher risk of type 2 diabetes mellitus (T2DM),[1,2] but these studies are difficult to interpret because of the difficulty of distinguishing between correlated measures of liver function and the possibility of confounding by poor health causing both poor liver function and T2DM.[3] Recently, Mendelian randomization (MR) studies have clarified that higher alanine aminotransferase (ALT) rather than other measures of liver function, could play a role in T2DM.[4,5] Adiposity is also a very well-established cause of T2DM.[6] Whether poor liver function also causes adiposity and thereby contributes to T2DM is unclear. Observationally, poor liver function is associated with obesity,[7,8] but these studies are open to confounding by lifestyle, including diet[9] and physical activity,[10] health status and socioeconomic position (SEP).[11] As such, whether poor liver function is an additional contributor to the obesity epidemic remains uncertain.

To inform this question when experimental evidence is lacking, we conducted two complimentary analyses with different assumptions and study designs. Observationally, we examined the association of liver function (ALT, alkaline phosphatase (ALP)) with adiposity in young people in a setting with little clear socio-economic patterning of obesity, so as to reduce confounding by poor health and socio-economic position, i.e., in Hong Kong's "Children of 1997" birth cohort.[12] We also, for the first time, used an MR study design to assess the effects of liver enzymes on adiposity, which takes advantage of the random allocation of genetic endowment at conception thereby providing randomization analogous to the randomization in randomized controlled trials.[13] We assessed the associations of genetically predicted liver enzymes (ALT, ALP and gamma glutamyltransferase (GGT))[14] with adiposity indices, i.e., body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR), using the Genetic Investigation of ANthropometric Traits (GIANT) consortium.[15-17] We also considered sex-specific associations, where possible, because sex differences in circulating levels of endogenous sex hormones are associated with both adiposity[18] and fatty liver.[19]

Materials and methods:

The "Children of 1997" birth cohort

The "Children of 1997" birth cohort is a population-representative Chinese birth cohort (n=8,327) which included 88% of all births in Hong Kong from 1 April 1997 to 31 May 1997.[20] The study was initially established to examine the effects of second-hand smoke exposure and breastfeeding on health services utilization to 18 months. Participants were recruited at the first postnatal visit to any of the 49 Maternal and Child Health Centers in Hong Kong, which parents of all newborns were strongly encouraged to attend to obtain free preventive care and vaccinations for their child/children up to 5 years of age. Information, including parental characteristics (maternal age, paternal age, parental smoking and parental education) and infant characteristics (birth weight, gestational age and sex) were obtained from a self-administered questionnaire in Chinese at recruitment and subsequent routine visits. Parental occupation, type of housing and income were also recorded. In 2007, contact was re-established followed by three postal/telephone questionnaire surveys and a Biobank clinical follow-up at 16-18 years. At the Biobank clinical follow-up, as a compromise between cost and comprehensiveness, liver enzymes were assessed from plasma ALT (IU/L) and plasma ALP (IU/L) analyzed using the Roche Cobas C8000 System, a discrete photometric chemistry analyzer, with International Federation of Clinical Chemistry standardized method with pyridoxal phosphate and substrates of L-alanine and 2-oxoglutarate for ALT, and an optimized substrate concentration and 2-amino-2-methyl-1-propanol as buffer plus the cations magnesium and zinc for ALP. These analyses were conducted at an accredited laboratory serving a teaching hospital in Hong Kong. Height (cm), weight (kg) and waist and hip circumference (cm) were measured using standard protocols.

Children of 1997

Exposure - liver function

Liver function at ~17.5 years was assessed from plasma ALT (IU/L) and plasma ALP (IU/L).

Outcome - Adiposity

Adiposity was assessed from BMI (kg/m^2), WC (cm) and WHR, which represent different aspects of adiposity. Although these are not completely normally distributed, we present them in natural units for ease of interpretation, given interpretation was similar using a gamma distribution.

Mendelian randomization

Genetic associations with liver enzymes

Single nucleotide polymorphisms (SNPs) associated with plasma log transformed ALT, ALP and GGT at genome-wide significance (p-value<5×10^{-8}) adjusted for age and sex were obtained from the largest available genome-wide association study (GWAS) of plasma levels of liver enzymes comprising 61,089 adults (~86% European, mean age 52.8 years, 50.6% women).[14,21] For SNPs in linkage disequilibrium (R^2>0.01), we retained SNPs with the lowest p-value using the *Clumping* function of MR-Base (TwoSampleMR) R package, based on the 1000 Genomes catalog.[22] Whether any of the selected SNPs was related to adiposity directly rather than through liver enzymes (pleiotropic effects) was assessed from their known phenotypes obtained from comprehensive curated genotype to phenotype cross-references, i.e., Ensembl (http://www.ensembl.org/index.html) and the GWAS Catalog (https://www.ebi.ac.uk/gwas/). We also identified SNPs from highly pleiotropic genes, such as *ABO* and *GCKR*, whose full functionality is not yet clearly understood.

Genetic associations with adiposity

Overall genetic associations with BMI (standard deviation (SD) units) were obtained from 2018 GIANT and UK Biobank meta-analysis[16] (GIANTUKB) (n=681,275), a meta-analysis of the GIANT GWAS Anthropometric 2015 BMI[15] (mean age 56.0 years, 53.8% women, 95% European) with a newly conducted GWAS of UK Biobank (100% European).[16] Sex-specific genetic associations with BMI were from GIANT GWAS Anthropometric 2015 BMI[15] (n=339,224, mean age 56.0 years, 53.8% women, 95% European). Overall and sex-specific genetic associations with WC (SD units) and WHR (SD units) were obtained from the GIANT GWAS Anthropometric 2015 Waist[17] (n=224,459, mean age 54.5 years, 54.6% women, 63.6% European). The GIANT (GWAS Anthropometric 2015 BMI[16] and the GWAS Anthropometric 2015 Waist[18]) adjusted for age, age-squared, study-specific covariates in a linear model.[15,17] The UK Biobank adjusted for age, sex, recruitment centre, genotyping batch and 10 principal components.[16]

Statistical analysis

In the "Children of 1997" birth cohort, baseline characteristics were compared between cohort participants who were included and excluded using Cohen effect sizes,[23] which indicates the magnitude of difference between groups independent of sample size. Cohen effect sizes are usually categorized as 0.10 for small, 0.30 for medium and 0.50 for large when considering categorical variables. [23] The associations of adiposity indices with potential confounders were assessed using independent t-test or analysis of variance.

We assessed the associations of liver function with adiposity indices adjusted for potential confounders, i.e., household income, highest parental education, type of housing, highest parental occupation, second-

hand and maternal smoking and sex, using multivariable linear regression. We also assessed whether associations differed by sex from the relevant interaction term.

In the Mendelian randomization study, we estimated the strength of the genetic instruments from the *F*-statistic.[24] A higher *F*-statistic indicates lower risk of weak instrument bias.[24] We aligned SNPs for exposure and outcome on allele and effect allele frequency to ensure all SNPs, in particular palindromic SNPs, were aligned correctly. SNPs that could not be unequivocally aligned were replaced by proxies or dropped. SNPs predicting liver function that were not available for adiposity indices were replaced by highly correlated proxies (R^2>0.9). Potential proxy SNPs were obtained from the GWAS[14] and their correlations with other SNPs were obtained using LDlink.[25,26]

We obtained unconfounded estimates of the effects of liver enzymes on adiposity indices overall and by sex by combining SNP-specific Wald estimates (SNP-outcome association divided by SNP-exposure association) using inverse variance weighting (IVW) with random effects for 4+ SNPs, which assumes that balanced pleiotropy, and with fixed effects for 3 SNPs or fewer. We repeated the analysis excluding pleiotropic SNPs that might be associated with the relevant outcome directly rather than via liver enzymes. As a sensitivity analysis, we used a weighted median (WM) and MR-Egger regression. The WM may generate correct estimates when >50% of weight is contributed by valid SNPs.[27] MR-Egger generates correct estimates even when all the SNPs are invalid instruments as long as the instrument strength independent of direct effect assumption is satisfied.[28] A non-null intercept from MR-Egger indicates potential directional pleiotropy and invalid IVW estimates.[29] Heterogeneity was assessed using the I^2 statistic.[28]

All statistical analyses were conducted using R version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria). The R package MendelianRandomization[30] was used to generate the estimates.

Ethics approval

Ethical approval for the "Children of 1997" study was obtained from the University of Hong Kong, Hospital Authority Hong Kong West Cluster Joint Institutional Review Board. Ethical approval from an Institutional Review Board is not required for the MR study since it only uses publicly available summary data.

Results

Children of 1997

In the Biobank clinical follow-up, 3,460 adolescents of 6,850 potentially active follow-up participants took part (51% follow-up) of whom, 3,458 had at least one measure of BMI, WC or WHR, as shown in Figure 1. The 4,869 participants without adiposity measures were not different from the included participants in terms of sex, second-hand and maternal smoking exposure and SEP with relatively small Cohen effect sizes (<0.13) (Table A.1). The mean and SD of BMI, WC and WHR were 20.9 kg/m^2 (SD 3.5 kg/m^2), 72.3 cm (SD 9.2 cm) and 0.77 (SD 0.06). Boys had higher BMI, WC, and WHR than girls. Maternal smoking was associated with larger BMI and WC. SEP had little association with BMI, WC and WHR (Table 1).

Table 2 shows ALT was positively associated with BMI, WC and WHR adjusted for potential confounders. ALP was negatively associated with BMI, WC and WHR. The associations of ALP with BMI, WC and WHR differed by sex, with the inverse associations only evident in boys.

Table 1. Baseline characteristics by body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR) among participants in Hong Kong's "Children of 1997" birth cohort, Hong Kong, China, 1997 to 2016

Characteristics	BMI (kg/m²)				WC (cm)				WHR			
	No.	%	Mean (SD)	*P-value**	No.	%	Mean (SD)	*P-value**	No.	%	Mean (SD)	*P-value**
BMI	3457		20.9 (3.5)	-		-		-	-	-	-	-
WC (cm)	-	-	-	-	3453	-	72.3 (9.2)	-	-	-	-	-
WHR	-	-	-	-	-	-	-	-	3452	-	0.77 (0.06)	-
Sex	3457	-	-	<0.001	3453	-	-	<0.001	3452	-	-	<0.001
Girl	1716	49.6%	20.7 (3.3)	-	1713	49.6%	69.7 (7.8)	-	1712	49.6%	0.75 (0.05)	-
Boy	1741	50.4%	21.2 (3.8)	-	1740	50.4%	74.9 (9.7)	-	1740	50.4%	0.79 (0.07)	-
Unknown	0	-	-	-	0	-	-	-	0	-	-	-
Second-hand and maternal smoking exposure	3457	-	-	<0.001	3453	-	-	<0.01	3452	-	-	0.05
None	943	27.3%	20.6 (3.1)	-	943	27.3%	71.6 (8.3)	-	942	27.3%	0.77 (0.06)	-
Prenatal second-hand smoking	1280	37.0%	20.9 (3.7)	-	1278	37.0%	72.3 (9.5)	-	1278	37.0%	0.77 (0.06)	-
Postnatal second-hand smoking	961	27.8%	21.3 (3.8)	-	959	27.8%	73.0 (9.8)	-	959	27.8%	0.77 (0.07)	-
Maternal smoking	128	3.7%	21.5 (3.4)	-	128	3.7%	73.6 (9.4)	-	128	3.7%	0.78 (0.06)	-
Unknown	145	4.2%	20.6 (3.1)	-	145	4.2%	71.0 (7.8)	-	145	4.2%	0.76 (0.06)	-
Highest parental education level	3457	-	-	0.71	3453	-	-	0.39	3452	-	-	0.29
Grade<=9	991	28.7%	21.0 (3.8)	-	990	28.7%	72.6 (9.8)	-	990	28.7%	0.77 (0.07)	-
Grades 10-11	1489	43.1%	20.9 (3.4)	-	1486	43.0%	72.2 (8.8)	-	1485	43.0%	0.77 (0.06)	-
Grades>=12	961	27.8%	20.9 (3.5)	-	961	27.8%	72.3 (9.3)	-	961	27.8%	0.77 (0.06)	-
Unknown	16	0.5%	20.8 (2.0)	-	16	0.5%	69.2 (5.1)	-	16	0.5%	0.74 (0.04)	-
Highest parental occupation	3457	-	-	0.01	3453	-	-	0.07	3452	-	-	0.42
Ⅰ (unskilled)	99	2.9%	20.7 (3.1)	-	99	2.9%	71.6 (8.9)	-	99	2.9%	0.77 (0.06)	-
Ⅱ (semiskilled)	285	8.2%	21.3 (3.7)	-	284	8.2%	72.8 (9.3)	-	284	8.2%	0.77 (0.07)	-
Ⅲ M (semiskilled)	503	14.6%	20.8 (3.5)	-	503	14.6%	72.2 (9.4)	-	503	14.6%	0.77 (0.07)	-
Ⅲ NM (nonmanual skilled)	879	25.4%	21.0 (3.7)	-	878	25.4%	72.5 (9.4)	-	877	25.4%	0.77 (0.06)	-

IV (managerial)	440	12.7%	21.4 (3.8)	-	440	12.7%	73.3 (10.2)	-	440	12.7%	0.77 (0.07)	-
V (professional)	797	23.1%	20.7 (3.3)	-	797	23.1%	71.8 (8.7)	-	797	23.1%	0.77 (0.06)	-
Unknown	454	13.1%	20.7 (3.4)	-	452	13.1%	71.6 (8.3)	-	452	13.1%	0.77 (0.06)	-
Household income per head at recruitment	3457	-	-	0.12	3453	-	-	0.14	3452	-	-	0.45
First quintile	572	16.5%	20.8 (3.5)	-	571	16.5%	71.9 (9.0)	-	571	16.5%	0.77 (0.07)	-
Second quintile	616	17.8%	21.0 (3.7)	-	614	17.8%	72.4 (9.9)	-	614	17.8%	0.77 (0.07)	-
Third quintile	618	17.9%	21.2 (3.7)	-	617	17.9%	73.2 (9.6)	-	617	17.9%	0.77 (0.06)	-
Fourth quintile	631	18.3%	20.8 (3.4)	-	631	18.3%	72.1 (8.8)	-	631	18.3%	0.77 (0.06)	-
Fifth quintile	646	18.7%	20.8 (3.2)	-	646	18.7%	72.0 (8.6)	-	645	18.7%	0.77 (0.07)	-
Unknown	374	10.8%	21.1 (3.7)	-	374	10.8%	72.2 (9.6)	-	374	10.8%	0.77 (0.07)	-
Type of housing at recruitment	3457	-	-	0.18	3453	-	-	0.51	3452	-	-	0.47
Public	1448	41.9%	21.1 (3.6)	-	1445	41.8%	72.5 (9.4)	-	1445	41.9%	0.77 (0.07)	-
Subsidized home ownership scheme	545	15.8%	20.8 (3.6)	-	544	15.8%	72.0 (9.2)	-	544	15.8%	0.77 (0.06)	-
Private	1359	39.3%	20.9 (3.4)	-	1359	39.4%	72.3 (9.1)	-	1358	39.3%	0.77 (0.06)	-
Unknown	105	3.0%	20.5 (3.0)	-	105	3.0%	71.2 (7.5)	-	105	3.0%	0.76 (0.05)	-

* Two-side *P*-value from independent t-test or analysis of variance (ANOVA)

Table 2 Adjusted associations of liver function (alanine aminotransferase (ALT) and alkaline phosphatase (ALP)) with adiposity markers (body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR)) at ~17.5 years in the Hong Kong's "Children of 1997" birth cohort, Hong Kong, China

Liver enzyme	Outcome	Sex-adjusted		Sex interaction	Boys		Girls	
		Beta	95% CI	p-value	Beta	95% CI	Beta	95% CI
ALT (IU/L)	BMI (kg/m^2)	0.10	0.09 to 0.11	0.19	0.10	0.09 to 0.12	0.09	0.07 to 0.11
	WC (cm)	0.25	0.23 to 0.28	0.04	0.27	0.24 to 0.30	0.21	0.17 to 0.26
	WHR	0.0013	0.0012 to 0.0015	0.08	0.0014	0.0012 to 0.0016	0.0011	0.0008 to 0.0014
ALP (IU/L)	BMI (kg/m^2)	-0.018	-0.024 to -0.012	<0.001	-0.024	-0.031 to -0.017	0.006	-0.006 to 0.018
	WC (cm)	-0.03	-0.05 to -0.02	<0.001	-0.05	-0.07 to -0.03	0.04	0.01 to 0.07
	WHR	-0.0002	-0.0003 to -0.0001	0.002	-0.0002	-0.0004 to -0.0001	0.0001	-0.0001 to 0.0003

Adjustment: household income, highest parental education, type of housing, highest parental occupation, second-hand and maternal smoking

Mendelian randomization

Genetic variants

In total, 4 SNPs independently predicting ALT, 14 SNPs independently predicting ALP and 26 SNPs independently predicting GGT at genome-wide significance were obtained.[14] All the palindromic SNPs were aligned based on effect allele frequency (Table A.2), except for rs2073398 (*GGT1, GGTLC2*), predicting GGT, which was replaced by rs5751901 (R^2=0.95) for GIANTUKB. Rs6834314 (*HSD17B13, MAPK10*) predicting ALT and rs944002 (*EXOC3L4*) predicting GGT which were replaced in the GWAS Anthropometric 2015 Waist by rs13102451 (R^2=1.00) and rs2297067 (R^2=0.98). Two SNPs, rs516246 (*FUT2*) and rs8038465 (*CD276*) predicting GGT had rather different allele distributions for GGT and adiposity indices in GIANT (GWAS Anthropometric 2015 BMI[16] and the GWAS Anthropometric 2015 Waist[17]).They were dropped in a sensitivity analysis separately. No proxy SNP (R^2>0.9) of rs516246 could be found in GIANTUKB. (Table A.3).

Of the 4 SNPs predicting ALT, rs2954021 (*TRIB1*) predicted both ALT and ALP. Of the 14 SNPs predicting ALP, rs281377 (*FUT2*) is highly associated with resting metabolic rate; rs579459 is located in the *ABO* gene. Of the 26 SNPs predicting GGT, rs516246 (*FUT2*) is associated with obesity-related traits; rs1260326 (*GCKR*) is associated with Crohn's disease which might be associated with adiposity (Table A.4). The *F* statistics were 15 for ALT, 158 for ALP and 45 for GGT.

Genetic associations with BMI, WC and WHR

Genetically instrumented ALT was negatively associations with BMI, with the association more obvious for women. ALT was not associated with WC or WHR. Genetically instrumented ALP and GGT were not clearly associated with BMI, WC or WHR. Overall, there was little statistical evidence of pleiotropy as few MR-Egger intercepts differed from the null. Large I^2 were seen for most estimates, heterogeneity was most evident for ALP (Table 3-5).

Table 3: Estimates of the effect of genetically instrumented liver enzymes ALT, ALP and GGT (per 100% change in concentration)[14] on BMI (standard deviation)[15,16] using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs

		Men and women together using All-GIANTUKB					Men using GWAS Anthropometric 2015 BMI				Women using GWAS Anthropometric 2015 BMI			
Liver enzyme	Method	SNP	Beta	95% CI	MR-Egger Intercept p-value	I^2 (p-value)	Beta	95% CI	MR-Egger Intercept p-value	I^2 (p-value)	Beta	95% CI	MR-Egger Intercept p-value	I^2 (p-value)
ALT	IVW	4	-0.17	-0.33 to -0.01	-	-	-0.13	-0.39 to 0.13	-	-	-0.19	-0.34 to -0.04	-	-
		3	-0.14	-0.20 to -0.07	-	-	-0.08	-0.24 to 0.09	-	-	-0.18	-0.34 to -0.03	-	-
	WM	4	-0.13	-0.20 to -0.05	-	-	-0.06	-0.25 to 0.12	-	-	-0.19	-0.36 to -0.02	-	-
		3	-0.12	-0.20 to -0.05	-	-	-0.05	-0.23 to 0.13	-	-	-0.18	-0.36 to -0.01	-	-
	MR-Egger	4	0.02	-0.25 to 0.30	0.11	91.5% (0.01)	0.24	-0.09 to 0.58	0.01	91.8% (0.48)	-0.16	-0.48 to 0.15	0.85	91.5% (0.56)
		3	-0.09	-0.25 to 0.06	0.54	92.8% (0.47)	0.14	-0.26 to 0.53	0.25	93.4% (0.45)	-0.19	-0.57 to 0.19	0.96	93.2% (0.30)
ALP	IVW	14	-0.07	-0.18 to 0.04	-	-	0.03	-0.10 to 0.17	-	-	-0.08	-0.21 to 0.04	-	-
		13	-0.06	-0.16 to 0.05	-	-	0.05	-0.07 to 0.17	-	-	-0.08	-0.21 to 0.05	-	-
		11	-0.07	-0.23 to 0.09	-	-	-0.02	-0.22 to 0.18	-	-	-0.17	-0.33 to -0.01	-	-
	WM	14	-0.05	-0.11 to -0.001	-	-	0.07	-0.03 to 0.18	-	-	-0.06	-0.16 to 0.04	-	-
		13	-0.05	-0.10 to -0.001	-	-	0.07	-0.03 to 0.18	-	-	-0.06	-0.16 to 0.04	-	-
		11	-0.07	-0.18 to 0.04	-	-	-0.12	-0.33 to 0.09	-	-	-0.11	-0.29 to 0.07	-	-
	MR-Egger	14	0.03	-0.17 to 0.22	0.23	89.4% (<0.001)	0.15	-0.06 to 0.37	0.18	89.4% (0.01)	-0.04	-0.26 to 0.18	0.63	89.3% (0.002)
		13	-0.001	-0.19 to 0.19	0.50	89.9% (<0.001)	0.12	-0.09 to 0.33	0.39	89.7% (0.02)	-0.05	-0.28 to 0.19	0.74	89.7% (0.001)
		11	0.28	-0.24 to 0.79	0.17	77.8% (<0.001)	0.21	-0.49 to 0.92	0.50	69.3% (0.01)	0.01	-0.54 to 0.56	0.51	71.5% (0.10)
GGT	IVW	26/25	0.04	-0.01 to 0.08	-	-	0.01	-0.05 to 0.07	-	-	0.001	-0.06 to 0.07	-	-
		24/23	0.04	-0.003 to 0.09	-	-	0.02	-0.03 to 0.07	-	-	0.02	-0.05 to 0.08	-	-

WM	26/25	0.03	0.01 to 0.06	-	-	0.02	-0.05 to 0.09	-	-	-0.01	-0.07 to 0.06	-	-
	24/23	0.03	0.01 to 0.06	-	-	0.02	-0.05 to 0.09	-	-	-0.01	-0.07 to 0.06	-	-
MR-Egger	26/25	0.06	-0.05 to 0.17	0.65	91.4% (<0.001)	0.05	-0.09 to 0.19	0.47	91.3% (0.02)	0.04	-0.11 to 0.19	0.56	91.2% (<0.001)
	24/23	0.05	-0.06 to 0.15	0.97	91.7% (<0.001)	0.03	-0.10 to 0.15	0.96	91.9% (0.25)	-0.01	-0.16 to 0.14	0.67	91.8% (0.002)

Rs516246 (*FUT2*) predicting GGT is not available in GIANTUKB, 25 SNPs remained

Excluded SNPs predicting ALT: rs2954021 (*TRIB1*) when SNP=3; excluded SNPs predicting ALP: rs2954021 (*TRIB1*), when SNP=13; excluded rs281377 (*FUT2*) and rs579459 (*ABO*) in addition when SNP=11; excluded SNPs predicting GGT: rs516246 (*FUT2*), rs1260326 (*C2orf16, GCKR*) and rs8038465 (*CD276*) when SNP=23 from GWAS Anthropometric 2015 BMI projects, excluding SNPs: rs516246 (*FUT2*) and rs1260326 (*C2orf16, GCKR*) when SNP=24 from GIANTUKB.

SNP: single nucleotide polymorphism; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyltransferase; BMI: body mass index; IVW: Inverse Variance Weighted; WM: Weighted Median.

Table 4 Estimates of the effect of genetically instrumented liver enzymes ALT, ALP and GGT (per 100% change in concentration)[14] on WC[17] (standard deviation) using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs

			All					Men					Women			
Liver enzyme	Method	SNPs	Beta	95% CI	MR-Egger Intercept p- value	I^2 (p-value)		Beta	95% CI	MR-Egger Intercept p-value	I^2 (p-value)		Beta	95% CI	MR-Egger Intercept p-value	I^2 (p-value)
ALT	IVW	4	-0.06	-0.23 to 0.12	-	-		0.0001	-0.35 to 0.35	-	-		-0.08	-0.26 to 0.10	-	-
		3	-0.03	-0.18 to 0.11	-	-		0.08	-0.13 to 0.29	-	-		-0.10	-0.28 to 0.08	-	-
	WM	4	-0.06	-0.22 to 0.10	-	-		0.06	-0.17 to 0.29	-	-		-0.10	-0.29 to 0.10	-	-
		3	-0.04	-0.21 to 0.12	-	-		0.08	-0.15 to 0.31	-	-		-0.12	-0.32 to 0.08	-	-
	MR-	4	0.01	-0.41 to 0.44	0.72	91.4% (0.12)		0.35	-0.34 to 1.05	0.26	91.4% (0.07)		-0.23	-0.62 to 0.17	0.42	91.6% 0.29)
	Egger	3	-0.08	-0.70 to 0.54	0.87	93.0% (0.07)		0.06	-0.44 to 0.56	0.93	93.0% (0.37)		-0.20	-0.86 to 0.46	0.75	93.2% (0.12)
ALP	IVW	14	-0.02	-0.16 to 0.11	-	-		-0.02	-0.18 to 0.14	-	-		-0.03	-0.15 to 0.10	-	-
		13	-0.02	-0.15 to 0.12	-	-		-0.004	-0.15 to 0.15	-	-		-0.03	-0.16 to 0.10	-	-
		11	-0.13	-0.33 to 0.08	-	-		-0.09	-0.33 to 0.15	-	-		-0.15	-0.34 to 0.04	-	-
	WM	14	0.01	-0.08 to 0.10	-	-		0.002	-0.12 to 0.12	-	-		0.03	-0.09 to 0.14	-	-
		13	0.02	-0.07 to 0.10	-	-		0.01	-0.11 to 0.13	-	-		0.02	-0.09 to 0.14	-	-
		11	-0.13	-0.32 to 0.06	-	-		-0.19	-0.43 to 0.06	-	-		-0.06	-0.28 to 0.15	-	-
	MR-	14	0.10	-0.13 to 0.32	0.20	89.6% (<0.001)		0.14	-0.13 to 0.40	0.15	89.7% (0.003)		0.06	-0.16 to 0.28	0.33	89.5% (0.02)
	Egger	13	0.09	-0.14 to 0.32	0.27	90.0% (<0.001)		0.10	-0.15 to 0.36	0.31	90.0% (0.01)		0.08	-0.15 to 0.30	0.26	89.9% (0.02)
		11	0.27	-0.43 to 0.97	0.25	71.3% (<0.001)		0.47	-0.32 to 1.27	0.14	69.4% (0.03)		0.12	-0.53 to 0.78	0.39	72.3% (0.05)
GGT	IVW	26	-0.01	-0.05 to 0.03	-	-		-0.04	-0.10 to 0.03	-	-		0.01	-0.05 to 0.07	-	-

	23	0.003	-0.04 to 0.04	-	-	-0.02	-0.07 to 0.04	-	-	0.02	-0.04 to 0.08	-	-
WM	26	-0.04	-0.10 to 0.02	-	-	-0.01	-0.09 to 0.06	-	-	-0.002	-0.08 to 0.07	-	-
	23	-0.04	-0.09 to 0.02	-	-	-0.01	-0.09 to 0.06	-	-	-0.002	-0.08 to 0.07	-	-
MR-	26	0.05	-0.05 to 0.14	0.18	91.3% (0.30)	0.05	-0.10 to 0.19	0.23	91.9% (0.14)	0.05	-0.09 to 0.19	0.50	91.3% (0.03)
Egger	23	0.01	-0.09 to 0.10	0.91	91.9% (0.50)	0.02	-0.12 to 0.16	0.57	92.4% (0.40)	0.01	-0.14 to 0.15	0.84	91.8% (0.06)

Excluded SNPs predicting ALT: rs2954021 (*TRIB1*) when SNP=3; excluded SNPs predicting ALP: rs2954021 (*TRIB1*), when SNP=13; excluded rs281377 (*FUT2*) and rs579459 (*ABO*) in addition when SNP=11; excluded SNPs predicting GGT: rs516246 (*FUT2*), rs1260326 (*C2orf16*, *GCKR*) and rs8038465 (*CD276*) when SNP=23

SNP: single nucleotide polymorphism; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyltransferase; WC: waist circumference; IVW: Inverse Variance Weighted; WM: Weighted Median.

Table 5: Estimates of the effect of genetically instrumented liver enzymes ALT, ALP and GGT (per 100% change in concentration)[14] on WHR[17] (standard deviation) using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs

		All					Men				Women			
Liver enzyme	Method	SNP	Beta	95% CI	MR-Egger Intercept p-value	I^2 (p-value)	Beta	95% CI	MR-Egger Intercept p-value	I^2 (p-value)	Beta	95% CI	MR-Egger Intercept p-value	I^2 (p-value)
ALT	IVW	4	0.04	-0.09 to 0.18	-	-	0.09	-0.13 to 0.30	-	-	0.02	-0.24 to 0.27	-	-
		3	0.03	-0.11 to 0.17	-	-	0.14	-0.08 to 0.35	-	-	-0.04	-0.22 to 0.13	-	-
	WM	4	0.05	-0.10 to 0.20	-	-	0.12	-0.11 to 0.35	-	-	-0.02	-0.21 to 0.16	-	-
		3	0.04	-0.11 to 0.19	-	-	0.13	-0.10 to 0.36	-	-	-0.03	-0.21 to 0.16	-	-
	MR-Egger	4	-0.004	-0.29 to 0.28	0.70	91.8% (0.56)	0.29	-0.16 to 0.74	0.31	91.5% (0.33)	-0.19	-0.74 to 0.36	0.40	91.8% (0.08)
		3	0.08	-0.25 to 0.41	0.73	93.4% (0.58)	0.10	-0.41 to 0.61	0.88	93.2% (0.56)	0.06	-0.35 to 0.47	0.59	93.5% (0.71)
ALP	IVW	14	-0.03	-0.16 to 0.10	-	-	-0.11	-0.26 to 0.05	-	-	0.04	-0.11 to 0.19	-	-
		13	-0.03	-0.17 to 0.10	-	-	-0.10	-0.26 to 0.06	-	-	0.02	-0.12 to 0.17	-	-
		11	-0.11	-0.34 to 0.12	-	-	-0.08	-0.36 to 0.20	-	-	-0.11	-0.32 to 0.11	-	-
	WM	14	0.02	-0.06 to 0.10	-	-	-0.12	-0.25 to 0.00	-	-	0.12	0.01 to 0.24	-	-
		13	0.02	-0.06 to 0.10	-	-	-0.12	-0.24 to -0.001	-	-	0.12	0.01 to 0.23	-	-
		11	-0.002	-0.17 to 0.17	-	-	0.02	-0.23 to 0.27	-	-	0.02	-0.17 to 0.21	-	-
	MR-Egger	14	0.08	-0.14 to 0.30	0.23	89.8% (<0.001)	-0.04	-0.31 to 0.23	0.54	89.7% (0.002)	0.17	-0.09 to 0.43	0.21	89.4% (<0.001)
		13	0.10	-0.12 to 0.32	0.14	90.1% (<0.001)	-0.06	-0.34 to 0.23	0.69	90.1% (0.001)	0.22	-0.01 to 0.45	0.04	89.8% (0.01)
		11	0.35	-0.42 to 1.13	0.22	71.6% (<0.001)	0.70	-0.18 to 1.57	0.07	69.6% (0.01)	0.11	-0.64 to 0.86	0.56	72.2% (0.01)
GGT	IVW	26	0.03	-0.01 to 0.07	-	-	0.02	-0.03 to 0.08	-	-	0.04	-0.02 to 0.10	-	-
		23	0.03	-0.02 to 0.07	-	-	0.03	-0.03 to 0.09	-	-	0.03	-0.03 to 0.10	-	-

WM	26	-0.01	-0.06 to 0.05	-	-	0.02	-0.06 to 0.10	-	-	-0.01	-0.08 to 0.07	-	-
	23	-0.01	-0.07 to 0.05	-	-	0.02	-0.06 to 0.10	-	-	-0.02	-0.09 to 0.06	-	-
MR-Egger	26	0.03	-0.06 to 0.13	0.95	91.4% (0.22)	0.08	-0.05 to 0.21	0.31	92.0% (0.89)	0.01	-0.13 to 0.15	0.64	91.3% (0.02)
	23	0.04	-0.07 to 0.15	0.84	92.0% (0.16)	0.07	-0.06 to 0.21	0.50	92.5% (0.84)	0.03	-0.12 to 0.18	0.94	91.8% (0.03)

Excluded SNPs predicting ALT: rs2954021 (*TRIB1*) when SNP=3; excluded SNPs predicting ALP: rs2954021 (*TRIB1*), when SNP=13; excluded rs281377 (*FUT2*) and rs579459 (*ABO*) in addition when SNP=11; excluded SNPs predicting GGT: rs516246 (*FUT2*), rs1260326 (*C2orf16*, *GCKR*) and rs8038465 (*CD276*) when SNP=23.

SNP: single nucleotide polymorphism; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyltransferase; WHR: waist-hip ratio; IVW: Inverse Variance Weighted; WM: Weighted Median.

Discussion

This novel study used two different approaches, an observational study and an MR study, with different data sources, assumptions, and different unrelated sources of bias to assess the role of liver function in adiposity. We found the clearest evidence for ALT being inversely associated with BMI, perhaps particularly among women.

We used an observational design to assess the association of liver function with adiposity indices in adolescents and an MR design in adults. However, limitations exist. First, observational studies are open to residual confounding by factors such as diet[9] and physical activity,[10] which are hard to measure precisely and eliminate. Hong Kong, with a different confounding structure for adiposity, provides a valuable setting in which to triangulate the evidence and to verify observations from Western settings that are potentially confounded.[31] However, it remains difficult to disentangle correlated factors reliably in an observational study, which might explain discrepancies between observational and MR estimates. It is also possible that associations may vary by history and trajectory of economic development, which has been much more rapid in Hong Kong than in the populations of largely European descent usually included in genetic studies.[32] Second, ALT was lower than 10 IU/L (n=254) for 7.3% of the participants in "Children of 1997" and was fixed at 5 IU/L, which is unlikely to affect the estimates, because it was only below the limit of detection for a relatively small proportion of observations. Third, follow-up was incomplete, however, no major differences were found between the participants with and without adiposity indices (Table A.1). As such, selection bias from loss-to-follow-up is unlikely be a major concern. Fourth, strong assumptions are required for MR which are also hard to demonstrate empirically, specifically that the genetic instruments, are independent of confounders of the exposure-outcome association and are only associated with the outcome via the exposure. Although we are not certain of the exact function of the SNPs predicting liver enzymes, some of them are mainly expressed in the liver according to the Human Protein Altas (http://www.proteinatlas.org/), making a causal role plausible. Pleiotropic effects are possible, but estimates were similar after excluding potentially pleiotropic SNPs,

and MR-Egger did not provide statistical evidence of pleiotropy. Although the I^2 was large, it could be driven by the low number of SNPs. Estimates for ALP showed some heterogeneity although the MR-Egger regression did not show directional pleiotropy. Fifth, the overlap of the GWAS for liver enzymes with adiposity indices from GIANT consortium is ~ 17%, which is unlikely to cause weak instrument bias from any common underlying data structure. Sixth, liver enzymes represent different aspects of liver function: ALT is a marker of hepatocyte integrity, ALP and GGT are markers of cholestasis, but may not completely or only represent liver function.[33] Seventh, we assessed sex differences on the assumption that genetic predictors of liver function are similar for women and men, which we could not test empirically.[34]

Observationally, the positive associations of ALT with BMI, WC and WHR are consistent with most of the previous observational studies in both adolescents and adults.[35-42] Observationally, the negative associations of ALP with adiposity are consistent with a previous study among Australian adolescents,[43] but not with all studies,[44] although few such studies have been conducted. However, the estimates differed between the observational and MR designs, probably because of the difficulty of distinguishing between correlated measures of liver function and the possibility of confounding. To our knowledge, no previous MR study has assessed the association of liver function with adiposity.

One possible explanation for the finding of ALT potentially reducing BMI is that ALT reduces muscle mass rather than or as well as fat mass, given ALT was inversely associated with BMI but not with WHR or WC. The Korean Sarcopenic Obesity Study, a prospective cohort study, found ALT and NAFLD inversely associated with skeletal muscle mass index.[45] However, observationally ALT is not consistently associated with sarcopenia,[46] possibly because of selection bias and confounding in studies of NAFLD patients vulnerable to sarcopenia. However, ALT reducing muscle mass would be consistent with ALT increasing the risk of diabetes,[3] because low muscle mass is a potential cause of diabetes.[47,48]

Conclusion

Higher ALT, but not ALP or GGT, possibly reducing specifically BMI, but not WC or WHR suggests that ALT may reduce muscle mass rather than fat mass. Given, MR studies suggest, specifically ALT causes diabetes, whether ALT reduces specifically muscle mass and thereby causes diabetes should be investigated, because it would mean that muscle mass could be an attractive target of intervention to prevent diabetes.

Acknowledgments

The authors thank colleagues at the Student Health Service and Family Health Service of the Department of Health for their assistance and collaboration. They also thank late Dr. Connie O for coordinating the project and all the fieldwork for the initial study in 1997-1998.

Data on measures of adiposity (2018 GIANT and UK Biobank meta-analysis, GWAS anthropometric 2015 BMI, GWAS anthropometric 2015 waist) have been contributed by the Genetic Investigation of ANthropometric Traits (GIANT) consortium and have been download from https://portals.broadinstitute.org/collaboration/giant/index.php/Main_Page.

Financial support:

This work is a substudy of the "Children of 1997" birth cohort which was initially supported by the Health Care and Promotion Fund, Health and Welfare Bureau, Government of the Hong Kong SAR [HCPF grant 216106] and reestablished in 2005 with support from the Health and Health Services Research Fund, Government of the Hong Kong SAR, [HHSRF grant 03040771]; the Research Fund for the Control of Infectious Diseases in Hong Kong, the Government of Hong Kong SAR [RFCID grant 04050172]; the University Research Committee Strategic Research Theme (SRT) of Public Health, the University of Hong Kong. The Biobank clinical follow-up was partly supported by the WYNG Foundation.

References

1 Hazlehurst, J. M., Woods, C., Marjot, T., Cobbold, J. F. & Tomlinson, J. W. Non-alcoholic fatty liver disease and diabetes. *Metabolism: clinical and experimental* **65**, 1096-1108, doi:10.1016/j.metabol.2016.01.001 (2016).

2 Williams, K. H., Shackel, N. A., Gorrell, M. D., McLennan, S. V. & Twigg, S. M. Diabetes and Nonalcoholic Fatty Liver Disease: A Pathogenic Duo. *Endocrine Reviews* **34**, 84-129, doi:10.1210/er.2012-1009 (2013).

3 Xu, L. *et al.* Liver enzymes and incident diabetes in China: a prospective analysis of 10 764 participants in the Guangzhou Biobank Cohort Study. *Journal of epidemiology and community health* **69**, 1040-1044, doi:10.1136/jech-2015-205518 (2015).

4 Liu, J., Au Yeung, S. L., Lin, S. L., Leung, G. M. & Schooling, C. M. Liver Enzymes and Risk of Ischemic Heart Disease and Type 2 Diabetes Mellitus: A Mendelian Randomization Study. *Scientific reports* **6**, 38813, doi:10.1038/srep38813 (2016).

5 Nano, J. *et al.* Gamma-glutamyltransferase levels, prediabetes and type 2 diabetes: a Mendelian randomization study. *International journal of epidemiology* **46**, 1400-1409, doi:10.1093/ije/dyx006 (2017).

6 Swerdlow, D. I. Mendelian Randomization and Type 2 Diabetes. *Cardiovascular Drugs and Therapy* **30**, 51-57, doi:10.1007/s10557-016-6638-5 (2016).

7 Fabbrini, E., Sullivan, S. & Klein, S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology (Baltimore, Md.)* **51**, 679-689, doi:10.1002/hep.23280 (2010).

8 Rinella, M. E. Nonalcoholic fatty liver disease: a systematic review. *Jama* **313**, 2263-2273, doi:10.1001/jama.2015.5370 (2015).

9 Ishimoto, T. *et al.* High-fat and high-sucrose (western) diet induces steatohepatitis that is dependent on fructokinase. *Hepatology (Baltimore, Md.)* **58**, 1632-1643, doi:10.1002/hep.26594 (2013).

10 Marchesini, G., Petta, S. & Dalle Grave, R. Diet, weight loss, and liver health in nonalcoholic fatty liver disease: Pathophysiology, evidence, and practice. *Hepatology (Baltimore, Md.)* **63**, 2032-2043, doi:10.1002/hep.28392 (2016).

11 Major, J. M. *et al.* Local geographic variation in chronic liver disease and hepatocellular carcinoma: contributions of socioeconomic deprivation, alcohol retail outlets, and lifestyle. *Annals of epidemiology* **24**, 104-110, doi:10.1016/j.annepidem.2013.11.006 (2014).

12 Schooling, C. M., Yau, C., Cowling, B. J., Lam, T. H. & Leung, G. M. Socio-economic disparities of childhood Body Mass Index in a newly developed population: evidence from Hong Kong's 'Children of 1997' birth cohort. *Archives of disease in childhood* **95**, 437-443, doi:10.1136/adc.2009.168542 (2010).

13 Lawlor, D. A., Harbord, R. M., Sterne, J. A., Timpson, N. & Davey Smith, G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Statistics in medicine* **27**, 1133-1163, doi:10.1002/sim.3034 (2008).

14 Chambers, J. C. *et al.* Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nature genetics* **43**, 1131-1138, doi:10.1038/ng.970 (2011).

15 Locke, A. E. *et al.* Genetic studies of body mass index yield new insights for obesity biology. *Nature* **518**, 197-206, doi:10.1038/nature14177 (2015).

16 Yengo, L. *et al.* Meta-analysis of genome-wide association studies for height and body mass index in ~700,000 individuals of European ancestry. *bioRxiv* (2018).

17 Shungin, D. *et al.* New genetic loci link adipose and insulin biology to body fat distribution. *Nature* **518**, 187-196, doi:10.1038/nature14132 (2015).

18 Mongraw-Chaffin, M. L. *et al.* Association Between Sex Hormones and Adiposity: Qualitative Differences in Women and Men in the Multi-Ethnic Study of Atherosclerosis. *The Journal of clinical endocrinology and metabolism* **100**, E596-E600, doi:10.1210/jc.2014-2934 (2015).

19 Lazo, M. *et al.* (MESA AC 291) The Association of Endogenous Sex Hormones with Liver Fat - Multi-Ethnic Study of Atherosclerosis (MESA). *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **13**, 1686-1693.e1682, doi:10.1016/j.cgh.2014.12.033 (2015).

20 Schooling, C. M., Hui, L. L., Ho, L. M., Lam, T. H. & Leung, G. M. Cohort profile: 'children of 1997': a Hong Kong Chinese birth cohort. *Int J Epidemiol* **41**, 611-620, doi:10.1093/ije/dyq243 (2012).

21 Kahali, B., Halligan, B. & Speliotes, E. K. Insights from Genome-Wide Association Analyses of Nonalcoholic Fatty Liver Disease. *Seminars in liver disease* **35**, 375-391, doi:10.1055/s-0035-1567870 (2015).

22 Hemani, G. *et al.* MR-Base: a platform for systematic causal inference across the phenome using billions of genetic associations. *bioRxiv*, doi:10.1101/078972 (2016).

23 Cohen, J. *Statistical power analysis for the behavioral sciences*. (Academic Press, 1977).

24 Burgess, S., Davies, N. M. & Thompson, S. G. Bias due to participant overlap in two-sample Mendelian randomization. *Genetic Epidemiology* **40**, 597-608, doi:10.1002/gepi.21998 (2016).

25 Machiela, M. J. & Chanock, S. J. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics (Oxford, England)* **31**, 3555-3557, doi:10.1093/bioinformatics/btv402 (2015).

26 Machiela, M. J. & Chanock, S. J. LDassoc: an online tool for interactively exploring genome-wide association study results and prioritizing variants for functional investigation. *Bioinformatics (Oxford, England)*, doi:10.1093/bioinformatics/btx561 (2017).

27 Bowden, J., Davey Smith, G., Haycock, P. C. & Burgess, S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* **40**, 304-314, doi:10.1002/gepi.21965 (2016).

28 Burgess, S., Bowden, J., Fall, T., Ingelsson, E., & Thompson, S. G. Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. *Epidemiology (Cambridge, Mass.)* (2016).

29 Bowden, J., Smith, G. D., Haycock, P. C. & Burgess, S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genetic Epidemiology* **40**, 304-314, doi:10.1002/gepi.21965 (2016).

30 Yavorska, O. O. & Burgess, S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. *International journal of epidemiology* **46**, 1734-1739, doi:10.1093/ije/dyx034 (2017).

31 Lawlor, D. A., Tilling, K. & Davey Smith, G. Triangulation in aetiological epidemiology. *International journal of epidemiology* **45**, 1866-1886, doi:10.1093/ije/dyw314 (2016).

32 Schooling, C. M. & Leung, G. M. A socio-biological explanation for social disparities in non-communicable chronic diseases: the product of history? *Journal of epidemiology and community health* **64**, 941-949, doi:10.1136/jech.2008.086553 (2010).

33 Giannini, E. G., Testa, R. & Savarino, V. Liver enzyme alteration: a guide for clinicians. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* **172**, 367-379, doi:10.1503/cmaj.1040752 (2005).

34 van Beek, J. H. *et al.* The genetic architecture of liver enzyme levels: GGT, ALT and AST. *Behavior genetics* **43**, 329-339, doi:10.1007/s10519-013-9593-y (2013).

35 Park, J. H., Kim, S. H., Park, S. & Park, M. J. Alanine aminotransferase and metabolic syndrome in adolescents: the Korean National Health and Nutrition Examination Survey Study. *Pediatric obesity* **9**, 411-418, doi:10.1111/j.2047-6310.2013.00199.x (2014).

36 Kim, J. & Jo, I. Relationship between body mass index and alanine aminotransferase concentration in non-diabetic Korean adults. *European journal of clinical nutrition* **64**, 169-175, doi:10.1038/ejcn.2009.131 (2010).

37 Anderson, E. L. *et al.* The Prevalence of Non-Alcoholic Fatty Liver Disease in Children and Adolescents: A Systematic Review and Meta-Analysis. *PloS one* **10**, e0140908, doi:10.1371/journal.pone.0140908 (2015).

38 Xu, L. *et al.* Liver enzymes as mediators of association between obesity and diabetes: the Guangzhou Biobank Cohort Study. *Annals of epidemiology* **27**, 204-207, doi:https://doi.org/10.1016/j.annepidem.2016.11.002 (2017).

39 Labayen, I. *et al.* Liver enzymes and clustering cardiometabolic risk factors in European adolescents: the HELENA study. *Pediatric obesity* **10**, 361-370, doi:10.1111/ijpo.273 (2015).

40 Fall, T. *et al.* Age- and sex-specific causal effects of adiposity on cardiovascular risk factors. *Diabetes* **64**, 1841-1852, doi:10.2337/db14-0988 (2015).

41 Lawlor, D. A., Sattar, N., Smith, G. D. & Ebrahim, S. The associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase. *American journal of epidemiology* **161**, 1081-1088, doi:10.1093/aje/kwi125 (2005).

42 Wu, X. Y. *et al.* Higher waist-to-height ratio and waist circumference are predictive of metabolic syndrome and elevated serum alanine aminotransferase in adolescents and young adults in mainland China. *Public health* **126**, 135-142, doi:10.1016/j.puhe.2011.11.001 (2012).

43 Booth, M. L. *et al.* The population prevalence of adverse concentrations and associations with adiposity of liver tests among Australian adolescents. *Journal of paediatrics and child health* **44**, 686-691, doi:10.1111/j.1440-1754.2008.01407.x (2008).

44 Nannipieri, M. *et al.* Liver enzymes, the metabolic syndrome, and incident diabetes: the Mexico City diabetes study. *Diabetes care* **28**, 1757-1762 (2005).

45 Hong, H. C. *et al.* Relationship between sarcopenia and nonalcoholic fatty liver disease: The Korean Sarcopenic Obesity Study. *Hepatology (Baltimore, Md.)* **59**, 1772-1778, doi:10.1002/hep.26716 (2014).

46 Lee, Y. H. *et al.* Sarcopenia is associated with significant liver fibrosis independently of obesity and insulin resistance in nonalcoholic fatty liver disease: Nationwide surveys (KNHANES 2008-2011). *Hepatology (Baltimore, Md.)* **63**, 776-786, doi:10.1002/hep.28376 (2016).

47 Schooling, C. M. *et al.* Adolescent build and diabetes: the Guangzhou Biobank Cohort Study. *Annals of epidemiology* **21**, 61-66, doi:10.1016/j.annepidem.2010.08.010 (2011).

48 Hou, W. W., Tse, M. A., Lam, T. H., Leung, G. M. & Schooling, C. M. Adolescent testosterone, muscle mass and glucose metabolism: evidence from the 'Children of 1997' birth cohort in Hong Kong. *Diabetic medicine : a journal of the British Diabetic Association* **32**, 505-512, doi:10.1111/dme.12602 (2015).

Legends:

Table 1. Baseline characteristics by body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR) among participants in Hong Kong's "Children of 1997" birth cohort, Hong Kong, China, 1997 to 2016

Table 2. Adjusted associations of liver function (alanine aminotransferase (ALT) and alkaline phosphatase (ALP)) with adiposity markers (body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR)) at ~17.5 years in the Hong Kong's "Children of 1997" birth cohort, Hong Kong, China

Table 3. Estimates of the effect of genetically instrumented liver enzymes ALT, ALP and GGT (per 100% change in concentration) on BMI (standard deviation) using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs

Table 4. Estimates of the effect of genetically instrumented liver enzymes ALT, ALP and GGT (per 100% change in concentration) on WC (standard deviation) using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs

Table 5. Estimates of the effect of genetically instrumented liver enzymes ALT, ALP and GGT (per 100% change in concentration) on WHR (standard deviation) using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs

Table A.1. Baseline characteristics of the participants who were included (n=3,458) and excluded (n=4,869) in the analyses of the Hong Kong's "Children of 1997" birth cohort, Hong Kong, China, 1997 to 2016

Table A.2. Characteristics of palindromic single nucleotide polymorphisms (SNPs) in the exposure and outcome genome-wide association studies (GWAS)

Table A.1: Baseline characteristics of the participants who were included (n=3,458) and excluded (n=4,869) in the analyses of the Hong Kong's "Children of 1997" birth cohort, Hong Kong, China, 1997 to 2016

Characteristics	Included (n=3,458)		Excluded (n=4,869)		Cohen effect size Φ
	n	%	n	%	
Sex	3458	-	4869	-	0.08
Female	1717	49.70%	2196	45.10%	-
Male	1741	50.30%	2608	53.60%	-
Unknown	0	-	65	1.30%	-
Second-hand and maternal smoking exposure	3458	-	4869	-	0.09
None	943	27.30%	1232	25.30%	-
Prenatal second-hand smoking	1280	37.00%	1520	31.20%	-
Postnatal second-hand smoking	962	27.80%	1492	30.60%	-
Maternal smoking	128	3.70%	275	5.60%	-
Unknown	145	4.20%	350	7.20%	-
Highest parental education levels	3458	-	4869	-	0.12
Grade<=9	991	28.70%	1476	30.30%	-
Grades 10-11	1490	43.10%	1957	40.20%	-
Grades>=12	961	27.80%	1222	25.10%	-
Unknown	16	0.50%	214	4.40%	-
Highest parental occupation	3458	-	4869	-	0.07
I(unskilled)	99	2.90%	140	2.90%	-
II(semiskilled)	285	8.20%	441	9.10%	-
III M (semiskilled)	504	14.60%	711	14.60%	-
III NM (nonmanual skilled)	879	25.40%	1167	24.00%	-
IV (managerial)	440	12.70%	682	14.00%	-
V(professional)	797	23.00%	917	18.80%	-
Unknown	454	13.10%	811	16.70%	-
Household income per head at recruitment	3458	-	4869	-	0.07
First quintile	573	16.60%	878	18.00%	-

Second quintile	616	17.80%	868	17.80%	-
Third quintile	618	17.90%	811	16.70%	-
Fourth quintile	631	18.20%	788	16.20%	-
Fifth quintile	646	18.70%	794	16.30%	-
Unknown	374	10.80%	730	15.00%	-
Type of housing at recruitment	3458	-	4869	-	0.08
Public	1448	41.90%	2128	43.70%	-
Subsidized home ownership scheme	545	15.80%	580	11.90%	-
Private	1360	39.30%	1885	38.70%	-
Unknown	105	3.00%	276	5.70%	-

Φ Cohen effect sizes are usually categorized into 3 levels, Chi-square tests for categorical variables: 0.10 for small. 0.30 for medium, 0.50 for large. For categorical variables, Cohen's w effect size is calculated as $w = \sqrt{\sum (p0 - p1)^2 / p0}$, where p0 is the proportion in given by the null hypothesis and p1 is the proportion given the alternative hypothesis; $w = \sqrt{\chi 2 / N}$ where N is the total count of the included and excluded participants.[23]

Table A.2. Characteristics of palindromic single nucleotide polymorphisms (SNPs) in the exposure[14] and outcome genome-wide association studies (GWAS)[15-17]

Phenotype	SNP	Effect Allele	Other Allele	EAF	EAF_GIANT[15,17]	EAF_GIANTUKB[16]
ALT	rs10883437	T	A	0.64	0.62	0.61
ALT	rs738409	G	C	0.23	0.23	0.23
ALP	rs10819937	C	G	0.17	0.14	0.19
ALP	rs6984305	A	T	0.11	0.11	0.11
ALP	rs7186908	C	G	0.24	0.18	0.2
GGT	rs2073398	G	C	0.34	0.39	NA
GGT	rs754466	T	A	0.24	0.29	0.26
GGT	rs9913711	C	G	0.65	0.68	0.66

ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyltransferase

EAF: effect allele frequency obtained from Chambers et al., 2011[14]

EAF_GIANT: effect allele frequency obtained from GWAS Anthropometric 2015 BMI[15] and GWAS Anthropometric 2015 Waist[17]

EAF_ GIANTUKB: effect allele frequency obtained from the 2018 GIANT and UK Biobank meta-analysis[16]

NA: no available data in specific dataset

Table A.3: Characteristics of unequivocally aligned single nucleotide polymorphisms (SNPs) in the exposure[14] and outcome genome-wide association study (GWAS)[15-17]

Phenotype	SNP	EA	OA	EAF_E	Proxy	Source of proxy	R^2¶	EA_P	OA_P	EAF_P¶	EA_O	OA_O	EAF_O	Outcome dataset	Beta in outcome	Standard error in outcome
ALT	rs6834314	A	G	0.75	-	-	-	-	-	-	NA	NA	NA	WC-Female	NA	NA
					rs13102451	Exposure GWAS*	1.00/1.00	A	G	0.75/-	A	G	0.72	WC-Female	-0.0083	0.0061
GGT	rs944002	G	A	0.21	-	-	-	-	-	-	NA	NA	NA	WC-Male/WHR-Male	NA	NA
					rs2297067	Exposure GWAS*	0.98/1.00	T	C	0.24/0.19	T	C	0.2	WC-Male	-0.0008	0.0062
														WHR-Male	0.0062	0.0063
GGT	rs2073398	G	C	0.34	-	-	-	-	-	-	NA	NA	NA	GIANTUKB	NA	NA
					rs5751901	Exposure GWAS*	0.95/0.96	C	T	-/0.37	C	T	0.35	GIANTUKB	0.0046	0.0021
GGT	rs516246	T	C	0.47	-	-	-	-	-	-	NA	NA	NA	GIANTUKB	NA	NA
											T	C	0.54	GIANT	-	-
GGT	rs8038465	T	C	0.39	-	-	-	-	-	-	T	C	0.41	GIANTUKB	0.0029	0.0020
													0.57	GIANT	-	-

* Proxy SNPs were from the GWAS of Chambers et al., 2011[14]

¶ The front estimate was obtained from LDlink[25,26] and the following estimate was the GWAS from Chambers et al., 2011

WC-Male, WC-Female and WHR-Male are of the GWAS Anthropometric 2015 Waist[17]

GIANT is of GWAS Anthropometric 2015 BMI[15] and the GWAS Anthropometric 2015 Waist[17]

GIANTUKB is of the 2018 GIANT and UK Biobank meta-analysis[16]

NA: no available data in specific dataset;

WC: waist circumference, WHR: waist-hip ratio

EA: effect allele of the original SNP; OA: other allele of the original SNP; EAF_E: effect allele frequency of the original SNP in the exposure GWAS;

EA_P: effect allele of the proxy SNP; OA_P: other allele of the proxy SNP; EAF_P: effect allele frequency of the proxy SNP;

EA_O: effect allele of the proxy SNP in the outcome GWAS; OA_O: other allele of the proxy SNP in the outcome GWAS; EAF_O: effect allele frequency of the proxy SNP in the outcome GWAS

Table A.4 Single nucleotide polymorphisms (SNPs) with potential pleiotropic effects other than via the specific liver enzyme from Ensembl and from GWAS Catalog

Liver Enzyme	SNPs	Location	Gene nearby	Phenotype, disease and trait - Ensembl	Phenotype, disease and trait - GWAS Catalog	Potential pleiotropy
ALT	rs10883437	10q24	*CPN1*	-	-	-
ALT	rs2954021	8q24	*TRIB1*	HDL, LDL, TC, ALP, Lymphocyte percentage of white cells, Neutrophil percentage of white cells, Response to fenofibrate (triglyceride levels)	Triglyceride levels, ALP, LDL	+
ALT	rs6834314	4q22	*HSD17B13, MAPK10*	-	-	-
ALT	rs13102451 (proxy of rs6834314)	4chr	-	-	-	-
ALT	rs738409	22q13	*PNPLA3, SAMM50*	Nonalcoholic fatty liver disease(NFLD), Cirrhosis (alcohol related)	Cirrhosis (alcohol related), Nonalcoholic fatty liver disease	-
ALP	rs10819937	9q21	*ALDOB, C9orf125*	-	-	-
ALP	rs16856332	2q24	*ABCB11*	-	-	-
ALP	rs174601	11q12	*C11orf10, FADS1, FADS2*	Blood metabolite levels, TC, Gondoic acid (20:1n-9) levels, HDL, Red blood cell fatty acid levels, Trans fatty acid levels	Gondoic acid levels, Trans fatty acid levels, Red blood cell fatty acid levels, Blood metabolite levels	-
ALP	rs1883415	6p22	*ALDH5A1, GPLD1*	-	-	-
ALP	rs1976403	1p36.12	*ALPL, NBPF3*	-	-	-
ALP	rs2236653	11q.24	*ST3GAL4*	-	-	-
ALP	rs281377	19q13	*FUT2*	Resting metabolic rate	Yeast infection, Resting metabolic rate	+
ALP	rs2954021	8q24	*TRIB1*	TC, HDL, LDL, ALT, Lymphocyte percentage of white cells, Neutrophil percentage of white cells, Response to fenofibrate	Triglyceride levels, ALT, LDL	+

				(triglyceride levels)		
ALP	rs314253	17p13	*ASGR1, DLG4*	TC, LDL	LDL cholesterol levels, Total cholesterol	-
ALP	rs579459	9q34	*ABO*	Blood metabolite ratios, C-reactive protein levels , TC, Coronary Artery Disease, Ischemic stroke, Large artery stroke, E-Selectin, LDL, Red blood cell count, Red blood cell traits, Soluble E-selectin levels, Soluble levels of adhesion molecules, Urinary metabolites (H-NMR features),	Glycated hemoglobin levels, Total cholesterol, LDL, Soluble levels of adhesion molecules, Red blood cell count, Urinary metabolites (H-NMR features), Coronary artery disease, Coronary artery disease or large artery stroke, Coronary artery disease or ischemic stroke, Coronary heart disease, Red blood cell traits, Blood metabolite ratios	+
ALP	rs6984305	8p23	*PPP1R3B*	TC, HDL	-	-
ALP	rs7186908	16q22	*HPR, PMFBP1*	-	-	-
ALP	rs7267979	20p11	*ABHD12,GINS1, PYGB*	-	-	-
ALP	rs7923609	10q21	*JMJD1C, NRBF2*	Educational attainment	Educational attainment	-
GGT	rs10513686	3q26	*SLC2A2*	-	-	-
GGT	rs1076540	22q11.21	*MICAL3*	-	-	-
GGT	rs10908458	1q21	*DPM3, EFNA1, PKLR*	-	-	-
GGT	rs12145922	1p22	*CCBL2, PKN2*	-	-	-
GGT	rs1260326	2p23	*C2orf16, GCKR*	Blood metabolite levels, C-reactive protein levels, Triglyceride levels, Caffeine metabolism (plasma 1,7-dimethylxanthine (paraxanthine) to 1,3,7-trimethylxanthine (caffeine) ratio), Cardiovascular disease risk factors, TC, Chronic kidney disease, Coffee consumption , Crohn's disease , Fasting Glucose (More seen in http://www.ensembl.org)	Alcohol consumption, Triglyceride, Crohn's disease, Inflammatory bowel disease, Plasma lactate levels, Hypertriglyceridemia,Renal overload goutBlood metabolite levels, Gout, Non-albumin protein levels, Two-hour glucose challenge (More could be assessed in https://www.ebi.ac.uk/gwas/search?query=rs1260326)	+
GGT	rs12968116	2q37	*ATP8B1*	Body Height, Familial Intrahepatic Cholestasis	-	-
GGT	rs13030978	2q12	*MYO1B, STAT4*	-	-	-

GGT	rs1335645	1p13	*CEPT1*	-	-	-
GGT	rs1497406	1p36.13	*RSG1, EPHA2*	-	-	-
GGT	rs17145750	7q11	*MLXIPL*	Metabolite levels (lipoprotein measures), Platelet Count	Platelet count, Metabolite levels (lipoprotein measures)	-
GGT	rs2073398	22q11.23	*GGT1, GGTLC2*	-	-	-
GGT	rs5751901 (proxy of rs2073398)	22q11.23	*GGT1*	Protein quantitative trait loci	GGT, Hematological and biochemical traits, Cardiovascular disease risk factors	
GGT	rs2140773	2q37	*EFHD1, LOC100129166*	-	-	-
GGT	rs2739330	22q11.23	*DDT, DDTL, GSTT1, GSTT2B, MIF*	-	-	-
GGT	rs339969	15q21	*RORA*	-	-	-
GGT	rs4074793	5q11	*ITGA1*	-	-	-
GGT	rs4503880	18q21.32	*NEDD4L*	-	-	-
GGT	rs4547811	4q31	*ZNF827*	-	-	-
GGT	rs4581712	16q23	*DYNLRB2*	-	-	-
GGT	rs516246	16q23	*FUT2*	TC, Crohn's disease (time to surgery), Inflammatory bowel disease, Obesity-related traits	Crohn's disease, Inflammatory bowel disease, Obesity-related traits	+
GGT	rs6888304	5p15	*CDH6*	-	-	-
GGT	rs7310409	12q24	*HNF1A, C12orf27*	C-reactive protein, Pancreatic Cancer, Pancreatic Neoplasms	Pancreatic cancer, C-reactive protein	-
GGT	rs754466	10q23	*DLG5*	-	-	-
GGT	rs8038465	15q23	*CD276*	-	-	-
GGT	rs9296736	6p12	*MLIP*	-	-	-
GGT	rs944002	14q32	*EXOC3L4*	Mean platelet volume	Mean platelet volume	-
GGT	rs2297067	14chr	-	Platelet Count, Primary biliary cholangitis	Primary biliary cholangitis, Platelet count	-

	(proxy of rs944002)					
GGT	rs9913711	17q24	*FLJ37644, SOX9*	-	-	-

ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyltransferase

TC: total cholesterol; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol

Figure 1. Flowchart of the Hong Kong's "Children of 1997" birth cohort, Hong Kong, China, 1997 to 2016