The effect of liver enzymes on body composition: a Mendelian randomization study

Jun Xi Liu[1], Shiu Lun Au Yeung[1], Man Ki Kwok[1], June Yue Yan Leung[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

Abstract:

Background: Higher alanine transaminase (ALT) is positively associated with diabetes but inversely associated with body mass index (BMI) in Mendelian randomization (MR) studies, suggesting liver function may affect body composition. To clarify, we assessed the association of liver function with muscle and fat mass observationally with two-sample MR as a validation.

Methods: In the population-representative "Children of 1997" birth cohort, we used multivariable linear regression to assess the adjusted associations of ALT and alkaline phosphatase (ALP) (IU/L) at ~17.5 years with muscle mass (kg) and body fat percentage (%). Genetic variants predicting ALT, ALP and gamma glutamyltransferase (GGT) (100% change in concentration) were applied to fat-free and fat mass (kg) in the UK Biobank (n=~331,000) to obtain unconfounded estimates using MR.

Results: Observationally, ALT was positively associated with muscle mass (0.11, 95% confidence interval (CI) 0.10 to 0.12) and fat percentage (0.15, 95% CI 0.13 to 0.17). ALP was inversely associated with muscle mass (-0.03, 95% CI -0.04 to -0.02) and fat percentage (-0.02, 95% CI -0.03 to -0.01). Using MR, ALT was inversely associated with fat-free mass (-0.41, 95% CI -0.64 to -0.19) and fat mass (-0.58, 95% CI -0.85 to -0.30). ALP was not clearly associated with body composition. GGT was positively associated with fat-free (0.30, 95% CI 0.01 to 0.06) and fat mass (0.41, 95% CI 0.10 to 0.71).

Conclusion: ALT reducing fat-free mass provides a possible pathway for the positive association of ALT with diabetes, and suggests a potential target of intervention.

Keywords:

Epidemiology; Mendelian randomization; Liver enzymes; Body composition.

Introduction

Observationally, poorer liver function, particularly nonalcoholic fatty liver disease (NAFLD), is associated with a higher risk of type 2 diabetes mellitus.[1] Mendelian randomization (MR) studies have clarified that higher alanine aminotransferase (ALT) rather than other aspects of liver function, could be the relevant factor causing diabetes.[2-4] However, modifiable targets on the pathway from poor liver function to diabetes are unclear and worthy of exploration. Recently, an unpublished MR study found ALT inversely associated with body mass index (BMI), indicating higher ALT might reduce BMI (bioRxiv. doi:10.1101/404319). This finding appears to contradict observational studies which show adiposity associated with poor liver function.[5] Besides, using BMI as a proxy measure for adiposity might not be correct, because it cannot distinguish muscle mass from fat mass.[6] Nevertheless, ALT reducing the muscle mass component of BMI would be consistent with ALT increasing the risk of diabetes, given low muscle mass is a potential cause of diabetes.[7,8] Observationally, liver function is associated with muscle mass, although these studies are not always consistent.[9,10] These inconsistencies could be due to confounding by lifestyle, health status, and socioeconomic position (SEP), or to selection bias in studies conducted in patients.

To clarify the roles of liver enzymes, indicating liver function, in body composition in the absence of experimental evidence, we conducted two analyses with different assumptions and study designs. Observationally, we examined the associations of ALT and alkaline phosphatase (ALP) with commonly used measures of muscle mass, i.e., muscle mass and grip strength,[11] and fat percentage in young people in a setting with little socioeconomic patterning of obesity, so as to reduce confounding by poor health and SEP, i.e., in Hong Kong's "Children of 1997" birth cohort.[12] Given the difference in body composition by sex, we also examined whether the associations differed by sex because such differences are likely interpretable even when other associations are confounded.[13] To validate the impact of liver enzymes on body composition, taking advantage of the random allocation of genetic endowment to avoid confounding,[14] we also used an MR design to assess the effects of genetically predicted ALT, ALP and gamma glutamyltransferase (GGT)[15] on body composition (fat-free mass, grip strength and fat mass) from the UK Biobank.[16]

Materials and Methods

Observational study – Children of 1997

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.[17] 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 are 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) was obtained from a self-administered questionnaire in Chinese at recruitment and subsequent routine visits. Parental occupation, type of housing and income were also recorded.

At the Biobank clinical follow-up at age ~17·5 years, as a compromise between cost and comprehensiveness, liver enzymes were assessed from ALT and ALP, a marker of hepatocyte integrity and a marker of cholestasis.[18] These were 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. Body composition indices including muscle mass and fat percentage were measured using bioimpedance analysis (BIA) by a Tanita segmental body composition monitor (Tanita BC-545, Tanita Co., Tokyo, Japan). Grip strength was measured by the Takei T.K.K.5401 GRIP D handgrip dynamometer (Takei Scientific Instruments Co. Ltd, Tokyo, Japan).

Exposures - liver enzymes

Liver function at ~17·5 years was assessed from plasma ALT (IU/L) and ALP (IU/L).

Outcomes – Body composition

Muscle was assessed from muscle mass (kg) and dominant hand grip strength (kg). Fat mass was assessed from body fat percentage.

Mendelian randomization study

Exposure - genetic predictors of liver enzymes

Single nucleotide polymorphisms (SNPs) predicting 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).[15,19] 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.[20] Whether any of the selected SNPs were associated with potential confounders was assessed from their Bonferroni corrected associations with height, alcohol use (intake frequency and intake versus 10 years previously), smoking (current smoking and past smoking), education, financial situation, physical activity (moderate and vigorous physical activity), and age of puberty (menarche and voice breaking) in the UK Biobank.[16] (ALT, 10 traits × 4 SNPs, p-value<1×10^{-3}; ALP, 10 traits × 14 SNPs, p-value<3×10^{-4}; GGT, 10 traits × 26 SNPs, p-value<1×10^{-4}). Additionally, we assessed the pleiotropic effects (related to body compositions directly rather than through liver enzymes) of the selected SNPs 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/). Lastly, we considered SNPs in the *ABO* and *GCKR* genes as potentially pleiotropic SNPs because these genes have many different effects that could possibly affect body composition directly rather than via liver enzymes.

Outcome - genetic associations with body composition

Genetic associations with fat-free mass (kg), grip strength (kg) (left and right hand), and fat mass (kg) were obtained from UK Biobank (~331,000 people of genetically verified white British ancestry) where the associations were

obtained from multivariable linear regression adjusted for the first 20 principal components, sex, age, age-squared, the sex and age interaction and the sex and age-squared interaction.[16]

Statistical analyses

Observational analyses

In the "Children of 1997" birth cohort, baseline characteristics were compared between cohort participants who were included and excluded using chi-squared tests and Cohen effect sizes which indicate the magnitude of differences between groups independent of sample size. Cohen effect sizes are usually categorized as 0.20 for small, 0.50 for medium and 0.80 for large, but when considering categorical variables they are categorized as 0.10 for small, 0.30 for medium and 0.50 for large.[21] The associations of body composition with potential confounders were assessed using independent t-tests or analysis of variance (ANOVA). We assessed the associations of liver enzymes with body composition indices using multivariable linear regression, adjusted for household income, highest parental education, type of housing, highest parental occupation, second-hand and maternal smoking, height and sex. For a small proportion of the observations, ALT was lower than 10 IU/L (n=254) and was fixed at 5 IU/L. We also assessed whether associations differed by sex from the significance of interactions adjusted for the other potential confounding interactions by sex.

Mendelian randomization analyses

We assessed the strength of the genetic instruments based on the *F*-statistic, where a higher *F*-statistic indicates a lower risk of weak instrument bias.[22] All SNPs were aligned according to the effect allele frequency for both the exposure and outcome.

We obtained the effects of liver enzymes on body composition indices based on meta-analysis of SNP-specific Wald estimates (SNP-outcome association divided by SNP-exposure association) using inverse variance weighting (IVW)

with multiplicative random effects for 4+ SNPs, which assumes balanced pleiotropy, and zero average pleiotropic effect of variants, and with fixed effects for 3 SNPs or fewer. Heterogeneity was assessed using the I^2 statistic where a high I^2 may indicate the presence of invalid SNPs.[23] Power calculations were performed using the approximation that the sample size for Mendelian randomization equates to that of the same regression analysis with the sample size divided by the r^2 for genetic variant on exposure.[24] Differences by sex were also assessed.

Sensitivity analyses

First, we repeated the analyses excluding potentially pleiotropic SNPs and those associated with confounders in the UK Biobank. Second, we used a weighted median (WM) which may generate correct estimates as long as >50% of weight is contributed by valid SNPs.[25] Third, we used MR-Egger which generates correct estimates even when all the SNPs are invalid instruments as long as the instrument strength independent of direct effect (InSIDE) assumption, that the pleiotropic effects of genetic variants are independent of the instrument strength, is satisfied.[23] A non-null intercept from MR-Egger indicates potentially directional pleiotropy and an invalid IVW estimate.[25] Finally, as an additional check on the validity of the MR estimates, we used Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO), which precisely detects and corrects for pleiotropic outliers assuming >50% of the instruments are valid, balanced pleiotropy and the InSIDE assumption are satisfied. Ideally, it gives a causal estimate with less bias and better precision than IVW and MR-Egger additionally assuming ≤10% of horizontal pleiotropic variants.[26,27]

All statistical analyses were conducted using R version 3·4·2 (R Foundation for Statistical Computing, Vienna, Austria). The R packages *MendelianRandomization*[28] and *MRPRESSO*[27] were used to generate the estimates.

Results

Children of 1997

Of 8,327 initially recruited, 6,850 are contactable and living in Hong Kong, of whom 3,460 (51%) took part in the Biobank clinical follow-up. Of these 3,460, 3,455 had measures of muscle mass, grip strength or fat percentage, as shown in Figure 1. The mean and standard deviation (SD) of muscle mass, grip strength and fat percentage were 42.6kg (SD 8.8kg), 25.8kg (SD 8.3kg) and 21.7% (SD 8.8%). Boys had higher muscle mass and grip strength but lower fat percentage than girls, but body composition had little association with SEP (Table 1). There were some differences between participants included and excluded from the study, such as sex, second-hand and maternal smoking exposure, and SEP, but the magnitude of these differences was small (Cohen effect size <0.15) (Supplemental Table 1).

The associations of liver enzymes with muscle mass and fat percentage differed by sex (Table 2). ALT was more strongly positively associated with muscle mass and fat percentage in boys. ALT was not clearly associated with grip strength. ALP was inversely associated with muscle mass, fat percentage and grip strength in boys, whereas, ALP was unclearly associated with muscle mass but positively associated with fat percentage and grip strength in girls.

Mendelian randomization

Genetic instruments for liver enzymes

Altogether, 4 SNPs independently predicting ALT, 14 SNPs independently predicting ALP and 26 SNPs independently predicting GGT at genome-wide significance were obtained.[15] Palindromic SNPs were all aligned according to effect allele frequency (Supplemental Table 2). The *F* statistic and variance explained (r^2) were 15 and 0.001 for ALT, 158 and 0.035 for ALP, and 45 and 0.019 for GGT. As such the MR study had 80% power with 5% alpha to detect a difference of 0.15, 0.03 and 0.04 in fat-free mass and fat mass effect size for ALT, ALP, and GGT respectively.

One SNP, rs2954021 (*TRIB1*), predicting ALT was associated with potential confounders. Seven SNPs, rs174601 (*C11orf10, FADS1, FADS2*), rs2236653 (*ST3GAL4)*, rs281377 (*FUT2*), rs2954021 (*TRIB1*), rs579459 (*ABO*), rs6984305 (*PPP1R3B*) and rs7923609 (*JMJD1C, NRBF2*) predicting ALP were associated with potential confounders. Eight SNPs, rs10908458 (*DPM3*, *EFNA1*, *PKLR*), rs12145922 (*CCBL2*, *PKN2*), rs1260326 (*GCKR*), rs1497406 (*RSG1*, *EPHA2*), rs17145750 (*MLXIPL*), rs516246 (*FUT2*), rs7310409 (*HNF1A*, *C12orf27*) and rs754466 (*DLG5*), predicting GGT were associated with potential confounders in UK Biobank at Bonferroni corrected significance (Supplemental Table 3).

Among the 4 SNPs predicting ALT, rs2954021 (*TRIB1*) predicts both ALT and ALP. Among the 14 SNPs predicting ALP, rs281377 (*FUT2*) is highly associated with resting metabolic rate, rs579459 is located in the *ABO* gene whose impact is extensive but unclear. Among the 26 SNPs predicting GGT, rs12968116 (*ATP8B1*) is associated with body height, rs1260326 (*GCKR*) and rs516246 (*FUT2*) are associated with Crohn's disease which might be associated with body composition (Supplemental Table 3).

Mendelian randomization estimates

Table 3 shows similar inverse estimates of genetically predicted ALT with fat-free mass and fat mass from all methods and by sex, however, the confidence intervals included the null value. ALT was not clearly associated with grip strength. Nevertheless, using MR-PRESSO ALT was inversely associated with fat-free mass and fat mass.

Table 4 shows genetically predicted ALP was not clearly associated with fat-free mass, fat mass, or grip strength using any method or by sex.

Table 5 shows genetically predicted GGT was not clearly associated with fat-free mass, fat mass or grip strength, but after excluding potential pleiotropy the corrected MR-PRESSO estimates suggested a positive association with fat-free mass and fat mass, particularly in women. GGT was not clearly associated with grip strength, although the WM estimate gave positive associations in women.

Discussion

Using two different complimentary designs with different strengths and weaknesses, we examined the impact of liver enzymes on body composition. Although there were discrepancies between the observational and MR estimates, some associations of ALT and GGT with body composition were found.

These two study designs have contrasting limitations. Observational studies are open to residual confounding, possibly by diet, lifestyle, and physical activity, although smoking is rare and alcohol consumption is low in Hong Kong.[29-31] Disentangling correlated factors is also difficult in an observational study. Inevitably, follow-up was incomplete (51%), but participants with and without body composition indices were similar, making selection bias unlikely. We also identified some sex differences which are less open to confounding. Inaccessibility, cost, and exposure to low-dose radiation precluded the use of dual-energy X–ray absorptiometry The reliability of BIA measurements particularly of body fat could vary for many reasons[32] but unlikely with liver function, so any bias was likely towards the null. The discrepancy between the observational and MR estimates might be due to reverse causality in the cross-sectional setting, and other limitations of observational studies. Differences by race/ethnicity are also possible. Lack of relevant data in Chinese precludes examining this possibility. However, we would normally expect causal factors to act consistently unless we know of reasons why the relevance of the specific operating mechanism varies by race/ethnicity.[33] MR assumes the genetic instruments strongly predict the exposure, are not confounded, and are only linked with the outcome by affecting the exposure. The *F* statistics were all >10 suggesting weak instrument bias is unlikely. We repeated the analyses excluding SNPs potentially associated with confounders. We conducted several sensitivity analyses to assess potential pleiotropy statistically, such as MR-Egger and MR-PRESSO, but found no evidence of directional pleiotropy. The MR estimates were relatively small, which might not be clinically significant, but could be relevant at the population level and may provide etiological insights.[34] The MR analyses were mainly restricted to people of European ancestry. Given the distribution of body composition varies by ethnicity, it is possible that the drivers of body composition also vary by ethnicity. However, more parsimoniously, it is likely that the drivers of body composition are similar across populations but their relevance varies. Specifically, ALT is higher in Chinese than in Westerners[35-37] which might be relevant to the lower fat-free mass in Chinese than in Westerners,[38] although ethnic variation in both ALT and fat-free mass could just be due to chance. The use of summary statistics in the MR study, means we could not comprehensively assess the

differences by age, sex or by baseline levels of liver enzymes; but we assessed the differences by sex observationally. Replicating the MR study in a Chinese population would be very helpful. Liver enzymes might not completely or only represent liver function, for example ALT may be transitorily affected by physical exertion, but liver enzymes are widely used as a surrogate of liver function.[18] Here, SNPs associated with vigorous physical activity were excluded. Fat-free mass and muscle mass are not identical. Fat-free mass also includes organs, skin, bones and body water, but does not vary as much as muscle mass. Finally, some overlap of participants between the GWAS used is inevitable, however, any effect on the estimates is likely to be small.

These observations are similar to previous observational studies.[9,39,40] However, only some of the previous observations, i.e., higher ALT associated with lower fat-free mass[41] and higher GGT associated with adiposity[42,43] were confirmed using MR. Consistent with observational studies I also found some differences by sex.[39,40,43]

The association of higher ALT, a measure of hepatocyte integrity, with lower fat-free mass, possibly differing by sex, may be due to growth hormone (GH)/ insulin-like growth factor 1 (IGF-1) or sex hormones which are associated with chronic liver diseases and muscle mass.[44-47] Studies using IGF-1 gene knock out animal models suggest IGF-1 is associated with hyperinsulinaemia and muscle insulin insensitivity,[48-50] although whether GH/IGF-1 also specifically affects ALT and muscle mass overall or differentially by sex is unknown. Schooling et al. have previously suggested that lower levels of androgens might cause higher risk of diabetes via lower muscle mass[46] and poor liver function may reduce androgens,[47] consistent with the sex differences observed. Additionally, it is also consistent with statins usage which is associated with lower testosterone,[51] elevated aminotransferase levels,[52] and higher diabetes risk.[53] Etiologically, these findings are consistent with the evolutionary public health, i.e., growth and reproduction trading-off against longevity, which may inform the identification of interventions. Reasons for an inverse association of ALT with fat mass are unclear but are consistent with a previous MR study (bioRxiv. doi:10.1101/404319) showing ALT negatively associated with BMI using the same genetic variants predicting ALT applied to the 2018 GIANT and UK Biobank meta-analysis.

Conclusion

Higher ALT, representing hepatocyte integrity, might reduce fat-free mass and fat mass with differences by sex; whilst higher GGT, as a marker of cholestasis, might increase fat-free mass and fat mass. As such, our study provides some indications that lower fat-free mass may mediate the positive effect of ALT on diabetes risk, which requires confirmation in other studies.

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.

Funding

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.

Data availability

The data that support the findings of this study are available on request from the "Children of 1997" data access committee: aprmay97@hku.hk. The data are not publicly available due to the participants could be identifiable from such extensive data which would comprise participant privacy. The datasets analyzed during the current MR study are publicly available summary data. These datasets were derived from the public domain resources: a publicly available GWAS study https://www.nature.com/articles/ng.970 and the UK Biobank GWAS http://www.nealelab.is/blog/2017/9/11/details-and-considerations-of-the-uk-biobank-gwas.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (the University of Hong Kong, Hospital Authority Hong Kong West Cluster Joint Institutional Review Board) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approval from an Institutional Review Board is not required for the MR study since it only uses publicly available summary data. This article does not contain any studies with animals performed by any of the authors

Informed consent

Informed written consent was obtained from the parents/guardians or participant if 18 years or older before participation in the Clinical Follow-up.

Declaration of interests

The authors declare that they have no conflict of interest.

Author contributions

JX Liu conducted the literature review, the analysis and drafted the manuscript. SL Au Yeung and CM Schooling conceptualized ideas, designed and directed the analytic strategy and supervised the study from conception to completion, with assistance from GM Leung. MK Kwok, JYY Leung, and LL Hui had full access to all the data in the study and took responsibility for the integrity of the data. All the authors contributed to the interpretation of the data, critically revising the paper and approval of the final version.

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

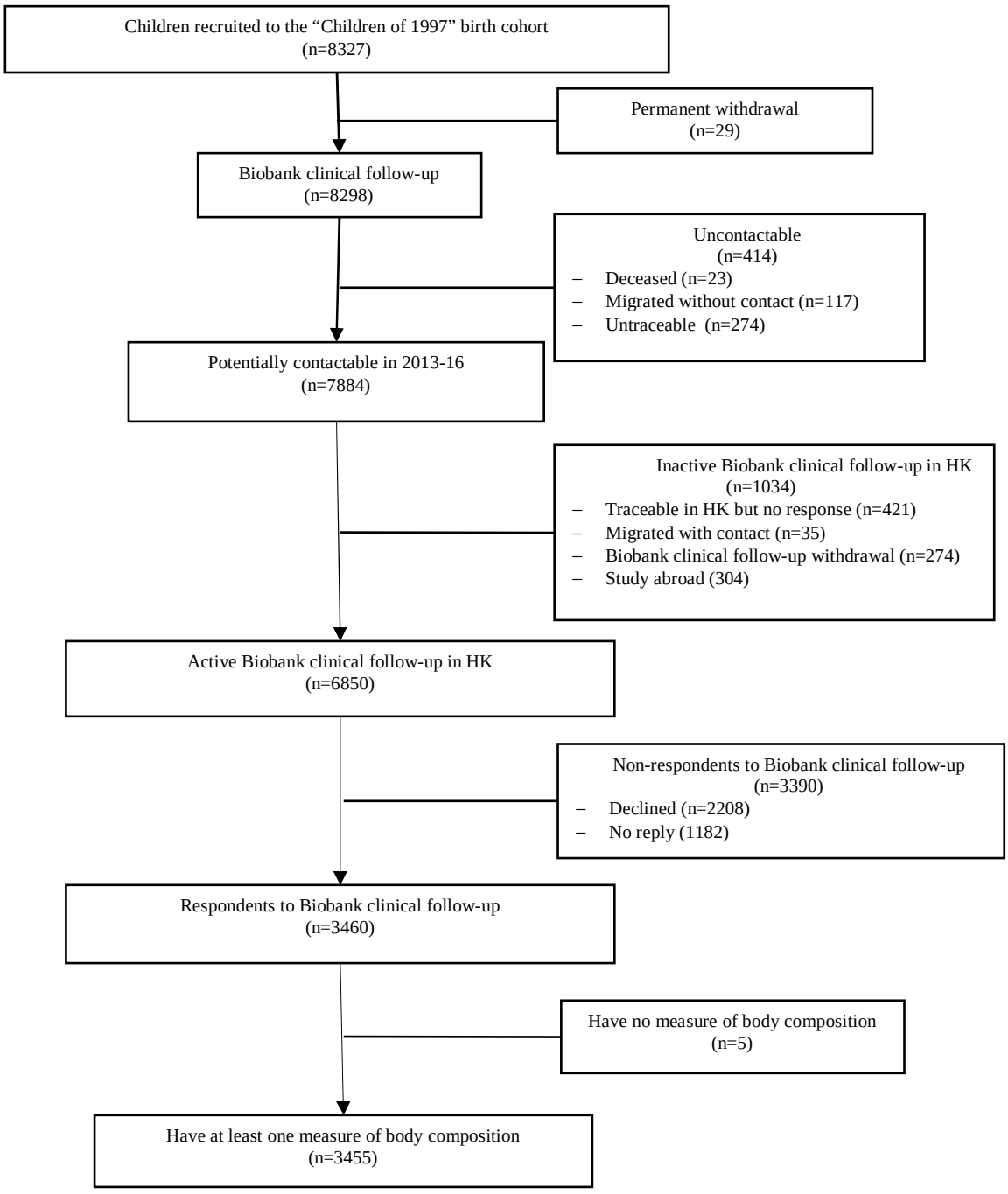

Table 1. Baseline characteristics muscle mass and fat percentage among participants in Hong Kong's "Children of 1997" birth cohort, Hong Kong, China, 1997 to 2016

Characteristics	Muscle mass (kg)				Grip strength				Fat percentage			
	No.	%	Mean (SD)	*P-value*	No.	%	Mean (SD)	*P-value*	No.	%	Mean (SD)	*P-value*
Muscle mass (kg)	3440		42.6 (8.8)									
Grip strength (kg)					3444		25.8 (8.3)					
Fat percentage (%)									3452		21.7 (8.8)	
Sex	3440			<0.001	3444			<0.001	3452			<0.001
Girl	1707	49.6%	35.3 (3.4)		1710	49.7%	19.9 (4.5)		1714	49.7%	28.1 (5.9)	
Boy	1733	50.4%	49.7 (6.3)		1734	50.3%	31.6 (7.0)		1738	50.3%	15.3 (6.4)	
Unknown	0	0.0%	-		0	0.0%	-		0	0.0%	-	
Second-hand and maternal smoking exposure	3440			0.07	3444			0.77	3452			0.17
None	940	27.3%	42.1 (8.4)		939	27.3%	25.6 (8.1)		943	27.3%	21.2 (8.5)	
Prenatal second-hand smoking	1275	37.1%	42.7 (8.8)		1276	37.0%	26.0 (8.4)		1276	37.0%	21.6 (9.0)	
Postnatal second-hand smoking	953	27.7%	43.0 (9.2)		956	27.8%	25.7 (8.3)		960	27.8%	22.0 (9.0)	
Maternal smoking	128	3.7%	42.7 (8.8)		128	3.7%	26.0 (8.2)		128	3.7%	22.9 (8.6)	
Unknown	144	4.2%	41.1 (8.6)		145	4.2%	25.3 (8.7)		145	4.2%	21.9 (9.0)	
Highest parental education level	3440			0.06	3444			0.12	3452			0.04
Grade<=9	984	28.6%	42.2 (9.1)		988	28.7%	25.4 (8.3)		989	28.7%	22.2 (9.0)	
Grades 10-11	1481	43.1%	42.4 (8.6)		1483	43.1%	25.7 (8.4)		1488	43.1%	21.6 (8.8)	
Grades>=12	959	27.9%	43.1 (8.9)		957	27.8%	26.3 (8.1)		959	27.8%	21.1 (8.7)	
Unknown	16	0.5%	39.7 (7.3)		16	0.5%	24.4 (6.8)		16	0.5%	23.9 (8.6)	
Highest parental occupation	3440			0.32	3444			0.04	3452			0.12
Ⅰ(unskilled)	98	2.8%	41.9 (9.3)		99	2.9%	25.4 (8.6)		99	2.9%	21.8 (8.1)	
Ⅱ(semiskilled)	281	8.2%	43.0 (9.0)		283	8.2%	26.4 (8.3)		285	8.3%	21.9 (8.8)	
Ⅲ (semiskilled)	503	14.6%	42.3 (9.0)		504	14.6%	25.1 (8.4)		503	14.6%	21.5 (8.8)	
Ⅲ (nonmanual skilled)	876	25.5%	42.4 (8.7)		878	25.5%	25.4 (8.1)		879	25.5%	22.2 (9.2)	
Ⅳ (managerial)	438	12.7%	43.2 (9.5)		438	12.7%	26.5 (8.5)		439	12.7%	22.2 (8.6)	
Ⅴ(professional)	794	23.1%	42.8 (8.5)		792	23.0%	26.2 (8.2)		795	23.0%	21.0 (8.5)	
Unknown	450	13.1%	42.0 (8.5)		450	13.1%	25.3 (8.4)		452	13.1%	21.5 (9.2)	
Household income per head at recruitment	3440			0.07	3444			0.16	3452			0.15
First quintile	566	16.5%	42.0 (8.5)		572	16.6%	25.6 (8.5)		571	16.5%	21.7 (8.9)	
Second quintile	613	17.8%	41.9 (9.3)		613	17.8%	25.0 (8.3)		616	17.8%	22.2 (8.7)	
Third quintile	616	17.9%	43.3 (8.8)		617	17.9%	26.1 (8.3)		618	17.9%	21.8 (9.1)	
Fourth quintile	630	18.3%	42.7 (8.9)		629	18.3%	25.9 (8.5)		630	18.3%	21.2 (8.7)	
Fifth quintile	644	18.7%	42.9 (8.6)		642	18.6%	26.1 (7.9)		645	18.7%	21.1 (8.5)	
Unknown	371	10.8%	42.6 (9.0)		371	10.8%	26.1 (8.3)		372	10.8%	22.2 (9.2)	
Type of housing at recruitment	3440			0.45	3444			0.44	3452			0.36
Public	1435	41.7%	42.5 (8.9)		1440	41.8%	25.8 (8.5)		1445	41.9%	21.9 (9.1)	
Subsidized home ownership scheme	545	15.8%	42.2 (8.8)		541	15.7%	25.2 (8.2)		544	15.8%	22.0 (8.9)	
Private	1355	39.4%	42.8 (8.8)		1358	39.4%	25.9 (8.1)		1358	39.3%	21.3 (8.5)	
Unknown	105	3.1%	41.8 (8.8)		105	3.0%	25.8 (8.7)		105	3.0%	21.2 (8.7)	

Table 2 Adjusted associations of liver function ALT and ALP with muscle mass, grip strength and fat percentage at ~17.5 years in the Hong Kong's "Children of 1997" birth cohort, Hong Kong, China

Exposure	Outcome	Sex-adjusted as confounder		p-value of interaction with sex	Boys		Girls	
		Beta	95% CI		Beta	95% CI	Beta	95% CI
ALT (IU/L)	Muscle mass (kg)	0.11	0.10 to 0.12	<0.001	0.13	0.11 to 0.14	0.06	0.04 to 0.07
	Grip strength (kg)	0.002	-0.01 to 0.02	0.73	0.002	-0.02 to 0.03	0.01	-0.02 to 0.04
	Fat percentage	0.15	0.13 to 0.17	0.19	0.16	0.14 to 0.18	0.13	0.09 to 0.16
ALP (IU/L)	Muscle mass (kg)	-0.03	-0.04 to -0.02	<0.001	-0.04	-0.05 to -0.03	-0.005	-0.015 to 0.005
	Grip strength (kg)	-0.01	-0.021 to -0.002	0.003	-0.02	-0.03 to -0.01	0.02	0.001 to 0.033
	Fat percentage	-0.02	-0.03 to -0.01	<0.001	-0.03	-0.04 to -0.02	0.03	0.004 to 0.048

Adjustment: adjusted for household income, highest parental education, type of housing, highest parental occupation, second-hand and maternal smoking, height and sex.
ALT: alanine aminotransferase; ALP: alkaline phosphatase

Table 3: Estimates of the effect of genetically instrumented (ALT (per 100% change in concentration) on fat-free mass, fat mass, and grip strength (left and right) using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs and potentially confounded SNPs

Outcome	Sex	SNP[a]	IVW				WM		MR-Egger			MR-PRESSO outlier corrected		
			Beta	95% CI	I2 (p-value)	Sex interaction p-value	Beta	95% CI	Beta	95% CI	Intercept p-value	Beta	95% CI	Sex interaction p-value
Fat-free Mass (kg)	All	4	-0.80	-2.41 to 0.81	91.4% (<0.001)	0.68	-0.45	-0.98 to 0.08	0.73	-2.61 to 4.07	0.31	-0.41	-0.64 to -0.19	0.46
		3	-0.42	-0.91 to 0.08	0.0% (0.81)	0.48	-0.43	-0.96 to 0.09	-0.75	-1.91 to 0.41	0.53	-	-	-
	Male	4	-1.10	-3.20 to 1.00	84.1% (<0.001)	-	-0.74	-1.69 to 0.21	0.30	-4.61 to 5.20	0.53	-0.62	-1.49 to 0.24	-
		3	-0.62	-1.49 to 0.25	0.0% (0.37)	-	-0.66	-1.59 to 0.28	-1.86	-3.92 to 0.19	0.19	-	-	-
	Female	4	-0.55	-1.87 to 0.76	85.3% (<0.001)	-	-0.13	-0.70 to 0.45	1.08	-1.10 to 3.25	0.10	-0.25	-0.70 to 0.20	-
		3	-0.25	-0.77 to 0.27	0.0% (0.48)	-	-0.12	-0.69 to 0.45	0.16	-1.07 to 1.40	0.47	-	-	-
Fat Mass (kg)	All	4	-1.22	-3.89 to 1.46	93.2% (<0.001)	0.90	-0.59	-1.38 to 0.19	1.41	-4.02 to 6.84	0.28	-0.58	-0.85 to -0.30	0.57
		3	-0.58	-1.30 to 0.15	0.0% (0.87)	0.60	-0.56	-1.33 to 0.22	-1.00	-2.71 to 0.71	0.60	-	-	-
	Male	4	-1.06	-4.14 to 2.01	91.1% (<0.001)	-	-0.49	-1.52 to 0.53	0.95	-6.24 to 8.13	0.53	-0.36	-1.58 to 0.85	-
		3	-0.36	-1.31 to 0.59	38.8% (0.20)	-	-0.35	-1.38 to 0.67	-2.24	-4.49 to 0.01	0.07	-	-	-
	Female	4	-1.34	-3.82 to 1.14	82.7% (<0.001)	-	-0.79	-1.94 to 0.37	1.82	-2.08 to 5.72	0.07	-0.76	-1.32 to -0.20	-
		3	-0.76	-1.83 to 0.31	0.0% (0.76)	-	-0.73	-1.88 to 0.42	0.09	-2.43 to 2.62	0.47	-	-	-
Left Hand Grip Strength (kg)	All	4	0.00	-0.57 to 0.57	0.0% (0.42)	0.30	-0.09	-0.74 to 0.56	0.09	-1.31 to 1.49	0.89	0.00[b]	-0.55 to 0.55	0.21
		3	0.08	-0.52 to 0.67	0.0% (0.39)	0.21	-0.06	-0.71 to 0.58	-0.34	-2.03 to 1.36	0.60	-	-	-
	Male	4	0.33	-0.65 to 1.32	0.0% (0.47)	-	0.27	-0.84 to 1.38	0.71	-1.51 to 2.94	0.70	0.33[b]	-0.57 to 1.23	-
		3	0.49	-0.53 to 1.51	0.0% (0.58)	-	0.30	-0.80 to 1.40	-0.09	-2.50 to 2.33	0.60	-	-	-
	Female	4	-0.30	-0.93 to 0.34	0.0% (0.79)	-	-0.41	-1.12 to 0.31	-0.47	-1.79 to 0.86	0.78	-0.30[b]	-0.67 to 0.08	-
		3	-0.29	-0.95 to 0.36	0.0% (0.59)	-	-0.40	-1.11 to 0.31	-0.60	-2.15 to 0.95	0.67	-	-	-
Right Hand Grip Strength (kg)	All	4	-0.03	-0.60 to 0.54	0.0% (0.64)	0.35	0.06	-0.57 to 0.70	0.48	-0.71 to 1.66	0.34	-0.03[b]	-0.46 to 0.40	0.25
		3	0.02	-0.57 to 0.61	0.0% (0.53)	0.20	0.14	-0.51 to 0.78	0.47	-0.93 to 1.86	0.49	-	-	-
	Male	4	0.26	-0.72 to 1.24	0.0% (0.48)	-	0.49	-0.61 to 1.58	1.39	-0.66 to 3.44	0.22	0.26[b]	-0.63 to 1.15	-
		3	0.44	-0.58 to 1.46	0.0% (0.71)	-	0.57	-0.53 to1.67	0.96	-1.45 to 3.36	0.64	-	-	-
	Female	4	-0.31	-0.95 to 0.33	0.0% (0.79)	-	-0.26	-0.97 to 0.45	-0.33	-1.67 to 1.00	0.96	0.31[b]	-0.69 to 0.07	-
		3	-0.36	-1.02 to 0.30	0.0% (0.72)	-	-0.27	-0.98 to 0.45	0.00	-1.56 to 1.56	0.62	-	-	-

Potentially pleiotropic and confounded SNP: rs2954021 (*TRIB1*)

a SNP= 4: all SNPs; SNP= 3, excluding rs2954021

b No outlier is found, presenting the raw estimate instead

ALT: alanine aminotransferase; IVW: inverse variance weighting; WM: weighted median; MR-PRESSO: Mendelian Randomization Pleiotropy RESidual Sum and Outlier

Table 4: Estimates of the effect of genetically instrumented ALP (per 100% change in concentration) on fat-free mass, fat mass, and grip strength (left and right) using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs and potentially confounded SNPs

Outcome	Sex	SNP[a]	IVW				WM		MR-Egger			MR-PRESSO outlier corrected		
			Beta	95% CI	I2 (p-value)	Sex interaction p-value	Beta	95% CI	Beta	95% CI	Intercept p-value	Beta	95% CI	Sex interaction p-value
Fat-free Mass (kg)	All	14	0.16	-0.66 to 0.97	87.9% (<0.001)	0.72	0.48	0.10 to 0.85	0.98	-0.44 to 2.40	0.17	0.33	-0.17 to 0.83	0.22
		11	-0.002	-0.98 to 0.97	81.9% (<0.001)	0.86	0.28	-0.34 to 0.90	1.26	-2.06 to 4.59	0.43	0.19	-0.45 to 0.83	0.98
		7	0.12	-1.20 to 1.43	86.5% (<0.001)	0.83	0.34	-0.33 to 1.01	1.21	-3.62 to 6.05	0.64	0.42	-0.04 to 0.87	0.54
	Male	14	0.29	-0.73 to 1.32	75.9% (<0.001)	-	0.80	0.12 to 1.47	1.65	-0.02 to 3.33	0.06	0.62	-0.03 to 1.27	-
		11	-0.09	-1.28 to 1.11	62.0% (<0.003)	-	0.17	-0.85 to 1.19	0.81	-3.35 to 4.96	0.66	-0.09	-0.93 to 0.75	-
		7	-0.01	-1.29 to 1.28	55.7% (0.04)	-	0.12	-1.00 to 1.24	0.43	-4.38 to 5.24	0.85	-0.01[b]	-1.29 to 1.28	-
	Female	14	0.04	-0.78 to 0.86	86.3% (<0.001)	-	0.03	-0.38 to 0.43	0.40	-1.11 to 1.92	0.58	0.12	-0.35 to 0.58	-
		11	0.07	-1.01 to 1.14	83.1% (<0.001)	-	-0.10	-0.84 to 0.64	1.63	-2.01 to 5.27	0.38	0.23	-0.51 to 0.97	-
		7	0.22	-1.29 to 1.73	88.4% (<0.001)	-	0.49	-0.28 to 1.26	1.86	-3.62 to 7.35	0.54	0.50	-0.44 to 1.44	-
Fat Mass (kg)	All	14	-0.62	-1.84 to 0.60	88.3% (<0.001)	0.77	-0.51	-1.08 to 0.05	0.38	-1.81 to 2.56	0.28	-0.53	-0.94 to -0.11	0.59
		11	-0.45	-1.95 to 1.05	83.3% (<0.001)	0.84	-0.48	-1.42 to 0.47	2.54	-2.32 to 7.41	0.21	-0.25	-0.96 to 0.46	0.86
		7	-0.35	-2.54 to 1.84	89.4% (<0.001)	0.94	-0.27	-1.37 to 0.84	4.61	-2.26 to 11.48	0.14	-0.05	-1.00 to 0.90	0.68
	Male	14	-0.46	-1.47 to 0.56	70.8% (<0.001)	-	-0.23	-0.95 to 0.48	0.54	-1.24 to 2.31	0.19	-0.24	-0.78 to 0.31	-
		11	-0.30	-1.14 to 0.55	10.0% (0.35)	-	-0.15	-1.21 to 0.92	1.25	-1.54 to 4.04	0.26	-0.30[b]	-1.14 to 0.55	-
		7	-0.43	-1.53 to 0.67	27.4% (0.22)	-	-0.62	-1.83 to 0.59	3.46	0.25 to 6.66	0.01	-0.43[b]	-1.53 to 0.67	-
	Female	14	-0.76	-2.38 to 0.85	85.3% (<0.001)	-	-0.73	-1.57 to 0.10	0.23	-2.71 to 3.18	0.43	-0.51	-1.33 to 0.30	-
		11	-0.58	-2.92 to 1.76	85.0% (<0.001)	-	-0.32	-1.76 to 1.12	3.67	-4.05 to 11.39	0.26	-0.44	-1.68 to 0.80	-
		7	-0.28	-3.64 to 3.08	90.2% (<0.001)	-	0.56	-1.00 to 2.12	5.63	-5.78 to 17.03	0.29	-0.02	-1.54 to 1.49	-
Left Hand Grip Strength (kg)	All	14	0.61	0.04 to 1.18	64.6% (<0.001)	0.66	0.93	0.41 to 1.44	1.48	0.59 to 2.37	0.02	0.76	0.29 to 1.22	0.59
		11	0.10	-0.66 to 0.85	56.1% (0.01)	0.35	0.08	-0.64 to 0.80	0.20	-2.45 to 2.85	0.94	0.38	-0.22 to 1.00	0.27
		7	0.13	-0.45 to 0.71	0.0% (0.59)	0.86	0.31	-0.45 to 1.07	0.91	-1.08 to 2.90	0.42	0.13[b]	-0.37 to 0.64	0.45
	Male	14	0.49	-0.31 to 1.28	44.9% (0.04)	-	1.30	0.46 to 2.13	2.11	1.05 to 3.17	0.00	0.69	0.03 to 1.35	-
		11	-0.26	-1.19 to 0.68	14.7% (0.30)	-	0.28	-0.91 to 1.47	1.59	-1.45 to 4.63	0.21	-0.26[b]	-1.19 to 0.68	-
		7	0.06	-0.94 to 1.07	0.0% (0.77)	-	0.32	-0.92 to 1.57	1.62	-1.82 to 5.07	0.35	0.06[b]	-0.68 to 0.81	-
	Female	14	0.72	0.09 to 1.36	64.2% (<0.001)	-	1.02	0.49 to 1.55	0.95	-0.23 to 2.13	0.65	0.75	0.26 to 1.24	-
		11	0.41	-0.60 to 1.42	69.7% (<0.001)	-	0.56	-0.35 to 1.47	-1.03	-4.45 to 2.39	0.39	0.44	-0.36 to 1.23	-
		7	0.20	-0.84 to 1.24	61.2% (0.02)	-	0.51	-0.50 to 1.51	0.25	-3.66 to 4.15	0.98	0.51	-0.37 to 1.39	-
Right Hand Grip Strength (kg)	All	14	0.46	-0.22 to 1.15	75.4% (<0.001)	0.55	1.32	0.74 to 1.90	1.58	0.54 to 2.62	0.01	-0.12	-0.81 to 0.58	0.001
		11	-0.23	-1.06 to 0.60	63.9% (0.002)	0.09	-0.39	-1.18 to 0.40	-0.28	-3.21 to 2.64	0.97	-0.12	-0.76 to 0.51	0.04
		7	-0.22	-0.94 to 0.50	35.1% (0.16)	0.11	-0.50	-1.33 to 0.34	0.63	-1.96 to 3.22	0.50	-0.22[b]	-0.94 to 0.50	0.11
	Male	14	0.27	-0.69 to 1.24	62.9% (<0.001)	-	0.56	-0.47 to 1.59	2.26	1.08 to 3.45	0.00	-1.02	-1.94 to -0.09	-
		11	-0.86	-1.88 to 0.16	28.9% (0.17)	-	-0.81	-2.01 to 0.40	0.44	-3.04 to 3.92	0.44	-0.86[b]	-1.88 to 0.16	-
		7	-0.82	-1.91 to 0.28	16.3% (0.31)	-	-0.71	-2.00 to 0.59	1.04	-2.70 to 4.78	0.31	-0.81[b]	-1.91 to 0.28	-
	Female	14	0.64	0.01 to 1.26	62.2% (0.001)	-	0.97	0.44 to 1.51	1.00	-0.14 to 2.13	0.45	0.70	0.22 to 1.18	-
		11	0.32	-0.62 to 1.27	64.7% (0.002)	-	0.72	-0.18 to 1.63	-0.94	-4.14 to 2.26	0.42	0.43	-0.28 to 1.14	-
		7	0.30	-0.52 to 1.12	37.2% (0.14)	-	0.67	-0.29 to 1.61	0.22	-2.87 to 3.31	0.96	0.30[b]	-0.52 to 1.12	-

Potentially pleiotropic SNPs: rs281377 (*FUT2*), rs2954021 (*TRIB1*) and rs579459 (*ABO*)

Potentially confounded SNPs: rs174601 (*C11orf10, FADS1, FADS2*), rs2236653 (*ST3GAL4*) rs281377 (*FUT2*), rs2954021 (*TRIB1*), rs579459 (*ABO*), rs6984305 (*PPP1R3B*) and rs7923609 (*JMJD1C, NRBF2*)

a SNP= 14: all SNPs; SNP= 11, excluding potentially pleiotropic SNPs; SNP= 7, excluding potentially pleiotropic SNPs and potentially confounded SNPs

b No outlier is found, presenting the raw estimate instead

ALP: alkaline phosphatase; IVW: inverse variance weighting; WM: weighted median; MR-PRESSO: Mendelian Randomization Pleiotropy RESidual Sum and Outlier

Table 5: Estimates of the effect of genetically instrumented GGT (per 100% change in concentration) on fat-free mass, fat mass, and grip strength (left and right) using Mendelian randomization with different methodological approaches with and without potentially pleiotropic SNPs and potentially confounded SNPs

Outcome	Sex	SNP[a]	IVW				WM		MR-Egger			MR-PRESSO outlier corrected		
			Beta	95% CI	I2 (p-value)	Sex interaction p-value	Beta	95% CI	Beta	95% CI	Intercept p-value	Beta	95% CI	Sex interaction p-value
Fat-free Mass (kg)	All	26	-0.02	-0.63 to 0.58	94.7% (<0.001)	0.79	0.07	-0.16 to 0.30	0.35	-1.01 to 1.72	0.55	0.17	-0.08 to 0.42	0.30
		23	0.16	-0.34 to 0.66	91.7% (<0.001)	0.91	0.10	-0.13 to 0.33	-0.01	-1.14 to 1.12	0.75	0.17	-0.10 to 0.43	0.33
		17	0.33	-0.05 to 0.71	81.4% (<0.001)	0.97	0.18	-0.08 to 0.45	0.13	-0.67 to 0.93	0.59	0.30	0.01 to 0.60	0.64
	Male	26	-0.09	-0.90 to 0.71	90.7% (<0.001)	-	-0.01	-0.42 to 0.40	0.24	-1.59 to 2.07	0.69	-0.03	-0.46 to 0.40	-
		23	0.13	-0.51 to 0.78	84.5% (<0.001)	-	0.01	-0.39 to 0.42	-0.19	-1.65 to 1.27	0.63	0.07	-0.35 to 0.49	-
		17	0.34	-0.25 to 0.93	75.9% (<0.001)	-	0.20	-0.25 to 0.64	0.01	-1.24 to 1.25	0.55	0.25	-0.16 to 0.67	-
	Female	26	0.04	-0.43 to 0.52	90.2% (<0.001)	-	0.14	-0.10 to 0.38	0.45	-0.61 to 1.52	0.39	0.21	-0.07 to 0.48	-
		23	0.18	-0.23 to 0.60	86.6% (<0.001)	-	0.15	-0.09 to 0.38	0.16	-0.79 to 1.11	0.95	0.32	0.06 to 0.58	-
		17	0.32	0.05 to 0.59	58.2% (0.001)	-	0.21	-0.05 to 0.46	0.24	-0.33 to 0.82	0.76	0.36	0.11 to 0.61	-
Fat Mass (kg)	All	26	0.22	-0.24 to 0.67	79.5% (<0.001)	0.30	0.22	-0.12 to 0.55	0.39	-0.64 to 1.41	0.71	0.11	-0.24 to 0.46	0.35
		23	0.27	-0.18 to 0.72	78.3% (<0.001)	0.30	0.22	-0.11 to 0.55	0.19	-0.84 to 1.23	0.87	0.04	-0.28 to 0.37	0.16
		17	0.45	-0.04 to 0.94	75.4% (<0.001)	0.49	0.24	-0.11 to 0.58	0.27	-0.76 to 1.30	0.70	0.41	0.10 to 0.71	0.17
	Male	26	-0.01	-0.52 to 0.51	73.0% (<0.001)	-	0.06	-0.37 to 0.49	0.14	-1.03 to 1.32	0.78	-0.03	-0.39 to 0.33	-
		23	0.06	-0.37 to 0.48	58.2% (<0.001)	-	0.06	-0.36 to 0.49	-0.11	-1.08 to 0.87	0.72	-0.01	-0.31 to 0.28	-
		17	0.30	-0.20 to 0.81	60.6% (<0.001)	-	0.09	-0.36 to 0.53	0.02	-1.04 to 1.09	0.56	0.23	-0.06 to 0.52	-
	Female	26	0.40	-0.17 to 0.97	72.0% (<0.001)	-	0.36	-0.12 to 0.83	0.60	-0.69 to 1.90	0.73	0.36	-0.13 to 0.84	-
		23	0.45	-0.16 to 1.07	74.1% (<0.001)	-	0.37	-0.10 to 0.84	0.45	-0.94 to 0.19	1.00	0.41	-0.11 to 0.93	-
		17	0.58	0.004 to 1.147	61.0% (<0.001)	-	0.41	-0.09 to 0.92	0.49	-0.73 to 1.70	0.87	0.65	0.12 to 1.18	-
Left Hand Grip Strength (kg)	All	26	0.06	-0.26 to 0.38	73.2% (<0.001)	0.71	0.17	-0.08 to 0.43	0.26	-0.47 to 0.98	0.56	0.19	-0.01 to 0.39	0.47
		23	0.09	-0.23 to 0.41	70.9% (<0.001)	0.81	0.18	-0.07 to 0.43	0.17	-0.55 to 0.90	0.81	0.17	-0.04 to 0.38	0.56
		17	0.22	-0.01 to 0.45	25.4% (0.16)	0.49	0.23	-0.05 to 0.51	0.25	-0.23 to 0.74	0.88	0.22[b]	-0.01 to 0.45	0.31
	Male	26	0.01	-0.42 to 0.43	53.3% (<0.001)	-	-0.01	-0.45 to 0.42	0.11	-0.85 to 1.07	0.82	0.15	-0.20 to 0.50	-
		23	0.06	-0.35 to 0.47	47.5% (0.007)	-	-0.02	-0.45 to 0.41	0.03	-0.90 to 0.97	0.96	0.07	-0.26 to 0.40	-
		17	0.13	-0.23 to 0.49	12.1% (0.31)	-	0.04	-0.43 to 0.50	0.19	-0.58 to 1.00	0.86	0.13[b]	-0.23 to 0.49	-
	Female	26	0.12	-0.23 to 0.47	71.8% (<0.001)	-	0.47	0.18 to 0.76	0.39	-0.40 to 1.18	0.44	0.25	-0.03 to 0.52	-
		23	0.13	-0.24 to 0.49	72.4% (<0.001)	-	0.47	0.18 to 0.77	0.31	-0.52 to 1.13	0.64	0.27	-0.0002 to 0.54	-
		17	0.30	-0.01 to 0.62	51.8% (0.007)	-	0.50	0.17 to 0.82	0.32	-0.36 to 1.00	0.97	0.3[b]	-0.01 to 0.62	-
Right Hand Grip Strength (kg)	All	26	0.01	-0.33 to 0.34	75.3% (<0.001)	0.65	0.16	-0.09 to 0.41	0.13	-0.63 to 0.89	0.72	0.14	-0.06 to 0.34	0.14
		23	0.04	-0.29 to 0.36	72.2% (<0.001)	0.69	0.16	-0.09 to 0.41	0.09	-0.65 to 0.83	0.88	0.11	-0.09 to 0.32	0.45
		17	0.16	-0.04 to 0.35	0.0% (0.48)	0.34	0.17	-0.11 to 0.44	0.15	-0.26 to 0.57	1.00	0.15[b]	-0.04 to 0.34	0.33
	Male	26	-0.07	-0.49 to 0.36	54.4% (<0.001)	-	-0.11	-0.55 to 0.33	0.10	-0.87 to 1.07	0.71	-0.05	-0.38 to 0.28	-
		23	-0.03	-0.46 to 0.41	53.1% (0.002)	-	-0.11	-0.54 to 0.32	0.09	-0.90 to 1.07	0.80	0.11	-0.09 to 0.32	-
		17	0.04	-0.30 to 0.38	0.0% (0.55)	-	-0.11	-0.58 to 0.37	0.22	-0.48 to 0.92	0.56	0.04[b]	-0.29 to 0.36	-
	Female	26	0.07	-0.30 to 0.44	74.3% (<0.001)	-	0.42	0.13 to 0.71	0.16	-0.68 to 1.00	0.81	0.27	0.01 to 0.52	-
		23	0.09	-0.27 to 0.46	72.3% (<0.001)	-	0.40	0.11 to 0.69	0.10	-0.74 to 0.93	1.00	0.24	-0.03 to 0.51	-
		17	0.26	-0.04 to 0.56	46.3% (0.02)	-	0.36	0.04 to 0.68	0.11	-0.53 to 0.74	0.59	0.26[b]	-0.04 to 0.56	-

Potentially pleiotropic SNPs: rs12968116 (*ATP8B1*), rs1260326 (*GCKR*) and rs516246 (*FUT2*)

Potentially confounded SNPs: rs10908458 (*DPM3, EFNA1, PKLR*), rs12145922 (*CCBL2, PKN2*), rs1260326 (*GCKR*), rs1497406 (*RSG1, EPHA2*), rs17145750 (*MLXIPL*), rs516246 (*FUT2*), rs7310409 (*HNF1A, C12orf27*) and rs754466 (DL*G5*)

a SNP= 26: all SNPs; SNP=23, excluding potentially pleiotropic SNPs; SNP= 17, excluding potentially pleiotropic SNPs and potentially confounded SNPs

b No outlier is found, presenting the raw estimate instead

GGT: gamma glutamyltransferase; IVW: inverse variance weighting; WM: weighted median; MR-PRESSO: Mendelian Randomization Pleiotropy RESidual Sum and Outlier

Supplemental Table 1. Baseline characteristics of the participants who were included (n=3455) and excluded (n=4872) in the analyses of the Hong Kong's "Children of 1997" birth cohort, Hong Kong, China, 1997 to 2016

Characteristics	Included (n=3455)		Excluded (n=4872)		p-value[a]	Cohen effect size[b]
	n	Mean (SD) / %	n	Mean (SD) / %		
Sex	3455		4872		<0.001	0.08
Female	1716	49.67%	2197	45.09%		
Male	1739	50.33%	2610	53.57%		
Unknown	0		65	1.33%		
Second-hand and maternal smoking exposure	3455		4872		<0.001	0.09
None	943	27.29%	1232	25.29%		
Prenatal second-hand smoking	1278	36.99%	1522	31.24%		
Postnatal second-hand smoking	961	27.81%	1493	30.64%		
Maternal smoking	128	3.70%	275	5.64%		
Unknown	145	4.20%	350	7.18%		
Highest parental education levels	3455		4872		<0.001	0.12
Grade<=9	989	28.63%	1478	30.34%		
Grades 10-11	1489	43.10%	1958	40.19%		
Grades>=12	961	27.81%	1222	25.08%		
Unknown	16	0.46%	214	4.39%		
Highest parental occupation	3455		4872		<0.001	0.07
Ⅰ(unskilled)	99	2.87%	140	2.87%		
Ⅱ(semiskilled)	285	8.25%	441	9.05%		
Ⅲ (semiskilled)	504	14.59%	711	14.59%		
Ⅲ (nonmanual skilled)	879	25.44%	1167	23.95%		
Ⅳ (managerial)	439	12.71%	683	14.02%		
Ⅴ(professional)	797	23.07%	917	18.82%		
Unknown	452	13.08%	813	16.69%		
Household income per head at recruitment	3455		4872		<0.001	0.07
First quintile	572	16.56%	879	18.04%		
Second quintile	616	17.83%	868	17.82%		
Third quintile	618	17.89%	811	16.65%		
Fourth quintile	631	18.26%	788	16.17%		
Fifth quintile	646	18.70%	794	16.30%		
Unknown	372	10.77%	732	15.02%		
Type of housing at recruitment	3455		4872		<0.001	0.08
Public	1445	41.82%	2131	43.74%		
Subsidized home ownership scheme	545	15.77%	580	11.90%		
Private	1360	39.36%	1885	38.69%		
Unknown	105	3.04%	276	5.67%		

a Two-side *P*-value from chi-square tests

b 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.

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

Phenotype	SNP	Effect Allele	Other Allele	EAF_Exposure	EAF_Outcome
ALT	rs10883437	T	A	0.64	0.60
ALT	rs738409	G	C	0.23	0.22
ALP	rs10819937	C	G	0.17	0.19
ALP	rs6984305	A	T	0.11	0.12
ALP	rs7186908	C	G	0.24	0.20
GGT	rs2073398	G	C	0.34	0.32
GGT	rs754466	T	A	0.24	0.25
GGT	rs9913711	C	G	0.65	0.67

EAF: Effect allele frequency

Supplemental Table 3. Single nucleotide polymorphisms (SNPs) with potential pleiotropic effects other than via the specific liver enzyme from Ensembl and from GWAS Catalog and potential confounders from UK Biobank

Liver Enzyme	SNPs	Location	Gene nearby	Phenotype, disease and trait -Ensembl	Phenotype, disease and trait - GWAS Catalog	Potential confounders with Bonferroni correction[a]	Potential pleiotropy	Potentially confounded
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	Alcohol intake frequency (2.57E-5), Height (3.54E-11),	+	+
ALT	rs6834314	4q22	*HSD17B13, MAPK10*	-	-	-	-	-
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	Height (2.35E-10)	-	+
ALP	rs1883415	6p22	*ALDH5A1, GPLD1*	-	-	-	-	-
ALP	rs1976403	1p36.12	*ALPL, NBPF3*	-	-	-	-	-
ALP	rs2236653	11q.24	*ST3GAL4*	-	-	Height (4.86E-5)	-	+
ALP	rs281377	19q13	*FUT2*	Resting metabolic rate	Yeast infection, Resting metabolic rate	Alcohol intake frequency (2.52E-7), Alcohol intake verse 10 years previously (5.44E-5), Menache (4.46E-4), Height (1.45E-5)	+	+
ALP	rs2954021	8q24	*TRIB1*	TC, HDL, LDL, ALT, Lymphocyte percentage of white cells, Neutrophil percentage of white cells, Response to fenofibrate (triglyceride levels)	Triglyceride levels, ALT, LDL	Alcohol intake frequency (2.57E-5), Height (3.54E-11),	+	+
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	Height (7.46E-6)	+	+
ALP	rs6984305	8p23	*PPP1R3B*	TC, HDL	-	Current tobacco smoking (1.12E-4)	-	+
ALP	rs7186908	16q22	*HPR, PMFBP1*	-	-	-	-	-
ALP	rs7267979	20p11	*ABHD12,GINS1,*	-	-	-	-	-

			PYGB					
ALP	rs7923609	10q21	*JMJD1C, NRBF2*	Educational attainment	Educational attainment	Menache (2.95E-8), Height (1.77E-11), Voice broke (7.33E-5)	-	+
GGT	rs10513686	3q26	*SLC2A2*	-	-	-	-	-
GGT	rs1076540	22q11.21	*MICAL3*	-	-	-	-	-
GGT	rs10908458	1q21	*DPM3, EFNA1, PKLR*	-	-	Past tabacco smoking (3.74E-5)	-	+
GGT	rs12145922	1p22	*CCBL2, PKN2*	-	-	Height (2.58E-35),	-	+
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)	Alcohol intake frequency (1.28E-43), Alcohol intake verse 10 years previously (1.54E-12), Height (7.79E-22), Voice broke (3.93E-4)	+	+
GGT	rs12968116	2q37	*ATP8B1*	Body Height, Familial Intrahepatic Cholestasis	-	-	+	-
GGT	rs13030978	2q12	*MYO1B, STAT4*	-	-	-	-	-
GGT	rs1335645	1p13	*CEPT1*	-	-	-	-	-
GGT	rs1497406	1p36.13	*RSG1, EPHA2*	-	-	Height (3.91E-5),	-	+
GGT	rs17145750	7q11	*MLXIPL*	Metabolite levels (lipoprotein measures), Platelet Count	Platelet count, Metabolite levels (lipoprotein measures)	Alcohol intake frequency (5.76E-7), Alcohol intake verse 10 years previously (4.70E-5)	-	+
GGT	rs2073398	22q11.23	*GGT1, GGTLC2*	-	-	-	-	-
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	Alcohol intake frequency (1.58E-9), Alcohol intake verse 10 years previously (4.40E-7), Menache (1.09E-4), Height (2.88E-7)	+	+
GGT	rs6888304	5p15	*CDH6*	-	-	-	-	-
GGT	rs7310409	12q24	*HNF1A, C12orf27*	C-reactive protein, Pancreatic Cancer, Pancreatic Neoplasms	Pancreatic cancer, C-reactive protein	Menache (5.51E-7), Voice broke (1.04E-5)	-	+
GGT	rs754466	10q23	*DLG5*	-	-	Height (2.57E-17)	-	+

GGT	rs8038465	15q23	*CD276*	-	-	-	-	-
GGT	rs9296736	6p12	*MLIP*	-	-	-	-	-
GGT	rs944002	14q32	*EXOC3L4*	Mean platelet volume	Mean platelet volume	-	-	-
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
a *P*-value with Bonferroni correction for ALT, ALP and GGT are 0.001, 0.0003 and 0.0001

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 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).

3 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).

4 De Silva, N. M. G. *et al.* Liver Function and Risk of Type 2 Diabetes: Bidirectional Mendelian Randomization Study. *Diabetes*, db181048, doi:10.2337/db18-1048 (2019).

5 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).

6 Nevill, A. M., Stewart, A. D., Olds, T. & Holder, R. Relationship between adiposity and body size reveals limitations of BMI. *American journal of physical anthropology* **129**, 151-156, doi:10.1002/ajpa.20262 (2006).

7 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).

8 Yeung, C. H. C., Au Yeung, S. L., Fong, S. S. M. & Schooling, C. M. Lean mass, grip strength and risk of type 2 diabetes: a bi-directional Mendelian randomisation study. *Diabetologia*, doi:10.1007/s00125-019-4826-0 (2019).

9 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).

10 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).

11 Bohannon, R. W. Muscle strength: clinical and prognostic value of hand-grip dynamometry. *Current opinion in clinical nutrition and metabolic care* **18**, 465-470, doi:10.1097/mco.0000000000000202 (2015).

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 VanderWeele, T. J. *Explanation in causal inference: methods for mediation*. (Oxford University Press, 2015).

14 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).

15 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).

16 Howrigan, D. *DETAILS AND CONSIDERATIONS OF THE UK BIOBANK GWAS*, <http://www.nealelab.is/blog/2017/9/11/details-and-considerations-of-the-uk-biobank-gwas> (September 20, 2017).

17 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. *International journal of epidemiology* **41**, 611-620, doi:10.1093/ije/dyq243 (2012).

18 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).

19 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).

20 Hemani, G. *et al.* The MR-Base platform supports systematic causal inference across the human phenome. *eLife* **7**, e34408, doi:10.7554/eLife.34408 (2018).

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

22 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).

23 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).

24 Freeman, G., Cowling, B. J. & Schooling, C. M. Power and sample size calculations for Mendelian randomization studies using one genetic instrument. *Int J Epidemiol* **42**, 1157-1163, doi:10.1093/ije/dyt110 (2013).

25 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).

26 Bowden, J., Davey Smith, G. & Burgess, S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *International journal of epidemiology* **44**, 512-525, doi:10.1093/ije/dyv080 (2015).

27 Verbanck, M., Chen, C.-Y., Neale, B. & Do, R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nature genetics* **50**, 693-698, doi:10.1038/s41588-018-0099-7 (2018).

28 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).

29 *Statistics on Youth Health-related Behaviour*, <https://www.chp.gov.hk/en/statistics/data/10/757/5522.html> (

30 Au, W. M. *et al.* Alcohol Drinking and Pro-drinking Practices in Parents of Hong Kong Adolescents. *Alcohol and Alcoholism* **49**, 668-674, doi:10.1093/alcalc/agu063 (2014).

31 HKSAR, H. K. C. a. S. D. o. (ed Hong Kong: Census and Statistics Department of HKSAR) (2018).

32 Dehghan, M. & Merchant, A. T. Is bioelectrical impedance accurate for use in large epidemiological studies? *Nutrition journal* **7**, 26-26, doi:10.1186/1475-2891-7-26 (2008).

33 Lopez, P., Subramanian, S. V. & Schooling, C. M. Effect measure modification conceptualized using selection diagrams as medication by mechanisms of varying population-level relevance. *J Clin Epidemiol*, doi:10.1016/j.jclinepi.2019.05.005 (2019).

34 Burgess, S., Butterworth, A., Malarstig, A. & Thompson, S. G. Use of Mendelian randomisation to assess potential benefit of clinical intervention. *BMJ : British Medical Journal* **345**, e7325, doi:10.1136/bmj.e7325 (2012).

35 Mu, R. *et al.* First Definition of Reference Intervals of Liver Function Tests in China: A Large-Population-Based Multi-Center Study about Healthy Adults. *PloS one* **8**, e72916, doi:10.1371/journal.pone.0072916 (2013).

36 Li, Y., Mussa, A. E., Tang, A., Xiang, Z. & Mo, X. Establishing reference intervals for ALT, AST, UR, Cr, and UA in apparently healthy Chinese adolescents. *Clinical biochemistry* **53**, 72-76, doi:https://doi.org/10.1016/j.clinbiochem.2018.01.019 (2018).

37 Zhang, G.-m. *et al.* Reference intervals for total bilirubin, ALT, AST and creatinine in healthy Chinese elderly. *Med Sci Monit* **20**, 1778-1782, doi:10.12659/MSM.892148 (2014).

38 Lear, S. A., Kohli, S., Bondy, G. P., Tchernof, A. & Sniderman, A. D. Ethnic Variation in Fat and Lean Body Mass and the Association with Insulin Resistance. *The Journal of Clinical Endocrinology & Metabolism* **94**, 4696-4702, doi:10.1210/jc.2009-1030 (2009).

39 Ruhl, C. E. & Everhart, J. E. Trunk fat is associated with increased serum levels of alanine aminotransferase in the United States. *Gastroenterology* **138**, 1346-1356, 1356.e1341-1343, doi:10.1053/j.gastro.2009.12.053 (2010).

40 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).

41 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).

42 Elshorbagy, A. K., Refsum, H., Smith, A. D. & Graham, I. M. The association of plasma cysteine and gamma-glutamyltransferase with BMI and obesity. *Obesity (Silver Spring, Md.)* **17**, 1435-1440, doi:10.1038/oby.2008.671 (2009).

43 Stranges, S. *et al.* Body fat distribution, relative weight, and liver enzyme levels: a population-based study. *Hepatology (Baltimore, Md.)* **39**, 754-763, doi:10.1002/hep.20149 (2004).

44 Guichelaar, M. M. & Charlton, M. R. Decreased muscle mass in nonalcoholic fatty liver disease: new evidence of a link between growth hormone and fatty liver disease? *Hepatology (Baltimore, Md.)* **59**, 1668-1670, doi:10.1002/hep.27058 (2014).

45 Cabrera, D. *et al.* Diet-Induced Nonalcoholic Fatty Liver Disease Is Associated with Sarcopenia and Decreased Serum Insulin-Like Growth Factor-1. *Digestive diseases and sciences* **61**, 3190-3198, doi:10.1007/s10620-016-4285-0 (2016).

46 Schooling, C. M., Au Yeung, S. L. & Leung, G. M. Why do statins reduce cardiovascular disease more than other lipid modulating therapies? *European journal of clinical investigation* **44**, 1135-1140, doi:10.1111/eci.12342 (2014).

47 Jaruvongvanich, V., Sanguankeo, A., Riangwiwat, T. & Upala, S. Testosterone, Sex Hormone-Binding Globulin and Nonalcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis. *Annals of hepatology* **16**, 382-394, doi:10.5604/16652681.1235481 (2017).

48 Sjogren, K. *et al.* Liver-derived IGF-I is of importance for normal carbohydrate and lipid metabolism. *Diabetes* **50**, 1539-1545 (2001).

49 Yakar, S. *et al.* Liver-specific igf-1 gene deletion leads to muscle insulin insensitivity. *Diabetes* **50**, 1110-1118 (2001).

50 Sandhu, M. S. Insulin-like growth factor-I and risk of type 2 diabetes and coronary heart disease: molecular epidemiology. *Endocrine development* **9**, 44-54, doi:10.1159/000085755 (2005).

51 Schooling, C. M., Au Yeung, S. L., Freeman, G. & Cowling, B. J. The effect of statins on testosterone in men and women, a systematic review and meta-analysis of randomized controlled trials. *BMC medicine* **11**, 57, doi:10.1186/1741-7015-11-57 (2013).

52 Jose, J. Statins and its hepatic effects: Newer data, implications, and changing recommendations. *Journal of pharmacy & bioallied sciences* **8**, 23-28, doi:10.4103/0975-7406.171699 (2016).

53 Crandall, J. P. *et al.* Statin use and risk of developing diabetes: results from the Diabetes Prevention Program. *BMJ Open Diabetes Research & Care* **5**, e000438, doi:10.1136/bmjdrc-2017-000438 (2017).