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Liver enzymes as mediators of association between obesity and diabetes: the Guangzhou Biobank Cohort Study

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Abstract

Purpose

To assess the proportion of the association between obesity and diabetes mediated by liver enzymes such as gamma-glutamyltransferase (GGT), alanine transaminase (ALT) and aspartate transaminase (AST).

Methods

Mediation analysis was used with adjustment for age, education, physical activity, smoking and alcohol use.

Results

9,748 participants from Phase 3 of the Guangzhou Biobank Cohort Study were recruited in 2006-2008. For women, the association of BMI and WC with glucose was partially mediated by GGT, 30% (95% confidence interval 23% to 40%) and 28% (23% to 34%), and by ALT, 15% (14% to 25%) and 14% (10% to 18%), respectively; for men, .the proportion mediated by GGT was 16% (9% to 26%) and 23% (12% to 36%) respectively, and by ALT 12% (4% to 22%) for BMI and for WC. The association of BMI and WC with glucose was not mediated by AST for women or men. Additionally considering of mediation by lipids did not change the mediation by GGT and ALT.

Conclusion

The effect of obesity on diabetes is partly mediated by GGT and ALT but not AST. There is no evidence of the mediation effect by lipids. Our results may provide opportunities to identify new targets for diabetes interventions. (200 words)

Introduction

The risk of diabetes is increased by obesity [1, 2] and poor liver function.[3]Weight loss improves liver function in obese individuals.[4]So, obesity may cause diabetes, via an impact on liver function. Little assessment has been made of the contribution of obesity to diabetes mediated through liver function. Traditional regression modeling is limited in identifying mediation. We took advantage of the recent improvement in structural equation modeling [5, 6] to estimate the risk of diabetes attributable to obesity mediated through poor liver function. Although this evidence is necessarily circumstantial, such an approach can still provide important support and motivation for more definitive investigations.

Mediation analysis allows researchers to identify mechanisms by which a treatment or exposure influences an outcome of interest, can provide evidence for the causal pathways by which obesity operate, and can be recommended as a method of improving the design and effectiveness of future diabetes prevention and interventions. As such, and given the high prevalence of diabetes in the world,[7] investigating the modifiable mediators of obesity that can be targeted may allow further effective control.

Methods

The Guangzhou Biobank Cohort Study (GBCS) is a study of lifestyle and genetic factors on health. Details of GBCS have been reported previously.[8]Briefly, GBCS is a 3-way collaboration of the Guangzhou 12th Hospital and the Universities of Hong Kong and Birmingham, UK. Participants were recruited in three phases from the "Guangzhou Health and Happiness Association for the Respectable Elders" (GHHARE), a community social and welfare organization. Membership is open to Guangzhou permanent residents aged 50 years or above for a nominal fee of 4 CNY (about 50 US cents) per month. GHHARE included about 7% of Guangzhou residents in this age group, with branches in all 10 districts of Guangzhou, the capital city of Guangdong province in southern China. Within sex and age group, the participants had fairly similar levels of chronic diseases such as diabetes and hypertension to nationally representative samples of urban Chinese.[8]

Physical examination included an interview concerning lifestyle, family and personal medical history and assessment of weight, height, waist circumference, blood pressure, fasting plasma glucose and lipids. Information on socioeconomic position and lifestyle including age, sex, education, smoking and alcohol use was collected by a standardized questionnaire. Physical activity was assessed using a validated Chinese version of the International Physical Activity Questionnaire.[9]Anthropometric measurements were performed by trained nurses in the Guangzhou 12th Hospital using standard protocols. Participants wore light clothing and no shoes. Body weight was measured to the nearest 0.1 kilogram using a balance beam scale (RGZ-120-RT, China). Waist circumference (WC) was measured horizontally around the smallest circumference between ribs and iliac crest, or at the navel, if there was no natural waistline. Body mass index (BMI) was calculated using measured weight and height as weight in kilograms divided by height in meters squared. Plasma glucose, lipids and liver enzymes (phase 3 only) were measured by Shimadzu CL-8000 Clinical Chemistry Analyzer (Shimadzu, Kyoto, Japan). The Guangzhou Medical Ethics Committee of the Chinese Medical Association approved the study and all participants gave written, informed consent before participation.

Statistical analysis

Pearson chi-square test and one-way analysis of variance were used to compare demographic and clinical characteristics by sex. We assessed whether the associations of BMI/WC with liver enzymes or diabetes varied by sex. BMI and WC were considered as exposures, liver enzymes including GGT, ALT and AST as potential mediators, and fasting plasma glucose as the study outcome. To assess the causal mediation effects, we used the potential outcomes framework. Let Mi(e) denote the potential values of the mediator for unit *i* under the exposure status (E_i=e), and Y_i(e, m) denote the potential outcome for unit i when E_i=e and M_i=m. Thus the observed variables can be written as M_i=M_i(E_i) and Y_i=Y_i(E_i, M_i(E_i)). The causal mediation effect ($\delta_i(e)$) for unit *i* under exposure level *e* can be obtained as $\delta_i(e) \equiv Y_i\big(\mathrm{e}, M_i(1)\big) - Y_i(\mathrm{e}, M_i(0))$. In this equation, the total effect can be expressed as $\delta_i(1)$ and the non-mediated effect as $\delta_i(0)$.

The average casual mediation effect was thus calculated as $\bar{\delta}_i(e) \equiv \mathbb{E}(\delta_i(e)) \equiv \mathbb{E}\{Y_i(\mathrm{e}, M_i(1)) - Y_i(\mathrm{e},\ M_i(0))\}$. [5, 6] Causal mediation analysis based on linear regression with a nonparametric bootstrap (i.e., 500 resamples) was conducted using R Mediation package [10]to obtain the adjusted associations of BMI/WC with liver enzymes, the adjusted associations of liver enzymes with fasting glucose, the adjusted association of BMI/WC with fasting glucose and the proportion mediated by liver enzymes. We also assessed if the association of BMI/WC with diabetes varied with liver enzymes. To facilitate comparison between liver enzymes, z-scores (standard deviations (SD)) of BMI, WC and log-transformed GGT, ALT and AST were used for data analysis. As both obesity and liver function may vary with age, socioeconomic position, smoking,[11]alcohol use[12, 13] and physical activity[14]; these were included as potential confounders. Given the association of obesity with liver enzymes might vary by sex, we assessed whether the association varied by sex from the heterogeneity across strata and the significance of interaction terms in a model which included potentially confounding interactions. As a sensitivity analysis we also considered multiple mediation and whether any associations were mediated by lipids. Statistical analyses were performed using R Studio (version 0.98.976) and specifically the "mediation" package for the mediation analysis and STATA/IC 10.1 (Stata Corp LP, College Station, TX, USA).

Results

A total of 9,748 participants recruited for the Phase 3 of the Guangzhou Biobank Cohort Study (GBCS) from September 2006 to January 2008 were included in the current study. Table 1 shows that men were older and had higher education, while women were more physically active and less likely to be alcohol drinkers or smokers. Men had higher WC and higher liver enzymes (GGT, ALT and AST), lower high- and low-density lipoprotein cholesterol but comparable BMI, triglycerides and fasting glucose to women.

As the association of obesity indices with liver enzymes varied by sex (p values for interaction <0.01), mediation analysis was conducted in women and men separately. In women, after adjustment for age,

education, physical activity, smoking and alcohol use, the associations of BMI and WC with fasting glucose were significantly mediated by GGT and ALT. In women, the proportion of the associations of BMI and WC with fasting glucose mediated by GGT was 30% (95% confidence interval (CI) 23% to 40%) and 28% (95% CI 23% to 34%), and mediated by ALT was 15% (95% CI 14% to 25%) and 14% (95% CI 10% to 18%), respectively. In men, the proportion of the associations of BMI and WC with fasting glucose mediated by GGT was lower than for women after similar adjustment (16% (95% CI 9% to 26%) and 23% (95% CI 12% to 36%), respectively). The same proportion of the associations of BMI and WC with glucose was mediated by ALT (both 12% (95% CI 4% to 22%). No mediation by AST was found for women or men.

Considering GGT as a main mediator and simultaneously taking into account ALT as an alternative mediator, compared with the model with single mediator, the mediation effect was reduced but remain statistically significant, and the regression coefficient decreased from 0.05 (95% CI 0.04 to 0.07) (Table 2) to 0.04 (95% CI 0.02 to 0.06) (Table not shown) for women, and from 0.04 (0.02 to 0.06) (Table 2) to 0.02 (-0.003 to 0.05) (Table not shown) for men. Additionally considering multiple mediation effect by including lipids such as triglycerides, and high- and low-density lipoprotein cholesterol in the causal mediation models did not reduce the average causal mediation effect by GGT and ALT. (Table not shown)

Discussion

The current study aimed to determine the degree to which liver enzymes, such as GGT, ALT and AST, mediate the known effect of obesity on glucose. We found that GGT most clearly mediated the effects of BMI/WC on fasting glucose, followed by ALT. As expected, AST did not mediate the effect of obesity on glucose. Mediation by GGT and ALT was greater in women than men. Questions about the effect of obesity on diabetes or of liver function on diabetes are not new; however, here we provide for the first time an assessment of the extent to which these effects of obesity on glucose are mediated by liver

enzymes based on a large community-based study and suggest a further target for diabetes prevention or intervention.

A recent study showed that obesity was associated with liver-specific increase of DNA methylation probably by increasing oxidative stress and metabolic pressure.[15]The authors suggest that obesity may increase the age of liver tissue and lead to liver-related comorbidities of obesity, such as insulin resistance and diabetes. Previous epidemiological studies have also reported an association of obesity and liver function with diabetes,[16, 17]but did not formally test mediation. We found that both GGT and ALT partially mediated the effect of BMI/WC on glucose, which may be more relevant at a population than an individual level. Given the mediation was partial, obesity may also have a direct effect on diabetes or an effect via other possible mediators.

In our study men had stronger BMI-ALT/GGT association than women (all P for sex interaction <0.01). The possible explanation of this finding is that women tend to have a higher proportion of body fat stored in subcutaneous regions while men tend to have more visceral fat.[18, 19] At the same BMI men may have a higher percentage of visceral fat than women,[18] which may lead to a higher risk for poor liver function.[20]Thus the statistical analysis was conducted separately by sex. Moreover, statistically, liver enzymes were considered as mediators rather than confounders because the causal association of adiposity with liver enzymes including ALT and GGT,[2] and the causal association of GGT with insulin resistance is supported by a recent study using Mendelian randomization approach.[3] The statistical method used in our study thus enabled us to provide more straightforward and robust estimate of the mediation effect,

The strengths of our study include the large sample size, the standardized assessments and the novelty of the analytic approach. However, there are some limitations of the current study. First, our models were based on measures of obesity indices and liver enzymes from a single point in time which might not

reflect risk associated with lifetime exposure to obesity or changes in liver enzymes over time. Measurement errors could attenuate the estimates of the mediation. Despite this, mediation by GGT and ALT were from moderate to low. Second, some of the effects of obesity is likely mediated by inflammation,[21]perhaps by C-reactive protein[22]or interleukin-6,[23] which were not available in our study. Further studies are warranted to evaluate mediation effect by inflammation markers. In addition, although BMI and WC are widely used for epidemiological studies, these obesity indices are somewhat suboptimal measures of adiposity because BMI is correlated both with obesity and muscle mass and both BMI and WC are of great inter-ethnic variability.[24, 25]

Conclusions

The effect of obesity on diabetes was partly mediated by GGT and ALT but not by AST. There is no evidence of the mediation effect due to lipids. This study re-emphasizes the importance of liver function for diabetes prevention and intervention, and offer opportunities to identify new target for diabetes interventions.

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Table 1: Demographic characteristics of the participants

	Women (n=7,274)	Men (n=2,474)	P-value
Age, years, mean (SD)	59.7 (7.6)	63.6 (7.6)	<0.001
Education, %			
Primary or below	41.0	28.7	<0.001
Middle school	52.8	56.4	
College or above	6.2	14.9	
Physical activity, %			
Inactive	8.4	9.0	<0.001
Moderate	25.0	34.1	
Active	66.6	56.9	
Alcohol use, %			
Never	59.2	38.9	<0.001
Former	4.9	6.6	
Current	35.9	54.6	
Smoking, %			
Never	97.3	37.3	<0.001
Former	1.3	26.9	
Current	1.5	35.7	
Body mass index, kg/m^2, mean (SD)	23.9 (3.4)	23.5 (3.2)	<0.001
Waist circumference, cm, mean (SD)	77.0 (8.9)	81.0 (9.2)	<0.001
HDL-cholesterol, mmol/l, mean (SD)	1.7 (0.4)	1.5 (0.4)	<0.001
LDL-cholesterol, mmol/l, mean (SD)	3.5 (0.7)	3.2 (0.7)	<0.001
Triglycerides, mmol/l, geometric mean (95% CI)	1.51 (1.5-1.53)	1.52 (1.49-1.56)	0.64
Log-GGT, u/l, mean (SD)	3.03 (0.56)	3.32 (0.58)	<0.001
Log-ALT, u/l, mean (SD)	3.06 (0.50)	3.15 (0.50)	<0.001
Log-AST, u/l, mean (SD)	3.23 (0.32)	3.28 (0.32)	<0.001
Fasting glucose, mmol/l, mean (SD)	5.7 (1.6)	5.7 (1.5)	0.19

SD=standard deviation; CI=confidence interval; HDL=high-density lipoprotein; LDL=low-density lipoprotein; GGT=gamma-glutamyltransferase; ALT= alanine transaminase; AST=aspartate transaminase

Table 2. Percent of the association of body mass index (BMI, kg/m^2) and waist circumference (WC, cm) with fasting glucose (mmol/l) mediated by gamma-glutamyltransferase(GGT, u/l), alanine transaminase (ALT, u/l) and aspartate transaminase (AST, u/l)

Exposures (E)	Mediators (M)	Average causal mediated effect	Non-mediated effect	Total effect	% of total effect mediated	P for E-M interaction
		β-coefficient (95% CI) †	β-coefficient (95% CI) †	β-coefficient (95% CI) †	% (95% CI)	
Women						
BMI	GGT	0.05 (0.04 to 0.07)	0.13 (0.09 to 0.17)	0.18 (0.15 to 0.22)	30% (23% to 40%)	0.03
BMI	ALT	0.03 (0.03 to 0.04)	0.15 (0.11 to 0.19)	0.19 (0.15 to 0.23)	15% (14% to 25%)	<0.001
BMI	AST	0.0001 (-0.0012 to 0.0015)	0.18 (0.14 to 0.21)	0.18 (0.14 to 0.21)	0.01% (-0.70% to 0.79%)	0.99
WC	GGT	0.08 (0.06 to 0.10)	0.20 (0.16 to 0.24)	0.28 (0.23 to 0.32)	28% (23% to 34%)	<0.001
WC	ALT	0.04 (0.03 to 0.05)	0.22 (0.18 to 0.26)	0.26 (0.22 to 0.30)	14% (10% to 18%)	<0.001
WC	AST	-0.001 (-0.003 to 0.0003)	0.24 (0.21 to 0.28)	0.24 (0.20 to 0.28)	-0.03% (-1.2% to 0.1%)	0.33
Men						
BMI	GGT	0.04 (0.02 to 0.06)	0.21 (0.14 to 0.28)	0.25 (0.18 to 0.32)	16% (9% to 26%)	0.14
BMI	ALT	0.03 (0.01 to 0.05)	0.23 (0.16 to 0.30)	0.26 (0.20 to 0.33)	12% (4% to 22%)	<0.001
BMI	AST	-0.0002 (-0.004 to 0.003)	0.25 (0.18 to 0.32)	0.25 (0.19 to 0.32)	-0.04% (-1.6% to 1.3%)	0.87
WC	GGT	0.05 (0.03 to 0.06)	0.16 (0.10 to 0.22)	0.20 (0.14 to 0.26)	23% (12% to 36%)	0.49
WC	ALT	0.02 (0.01 to 0.04)	0.18 (0.12 to 0.24)	0.20 (0.14 to 0.26)	12% (4% to 22%)	0.61
WC	AST	0.0006 (-0.002 to 0.004)	0.20 (0.14 to 0.26)	0.20 (0.14 to 0.26)	0.2% (-1.1% to 1.9%)	0.92

†: Adjusted for age, sex, education, physical activity, alcohol use and smoking.

*: All mediators or risk factors were standardized using Z-scores to facilitate comparison and interpretation.