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Elevated liver enzymes are associated with fasting plasma glucose levels among overweight and obese adults in Southern China: a cross-sectional study

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Abstract

Objective: The aim of this study was to determine the correlation between elevated liver enzymes and fasting plasma glucose levels (FPG) among overweight and obese adults who were compared with a control group of adults with normal weight.

Methods: In this cross-sectional study, 2915 individuals (≥18 years old) underwent real-time interviews and blood tests in 2014. Participants were divided into two groups, one was normal weight group, another one was overweight and obesity group..

Results: In normal weight group, there was no association of liver enzymes levels with FPG levels (alanine transaminase [ALT], P =0.519; aspartate aminotransferase.[AST], P =0.097)**.** However, adverse trends between liver enzymes levels and FPG levels were observed in overweight and obesity group (ALT, P =0.004; AST, P =0.023). After adjusting for confounding factors, the highest tertiles of ALT levels still remained significantly associated with FPG levels in 5.56 ≤ FPG < 7.00 mmol/L (odds ratio [OR] : 2.166, 95% confidence interval [CI]: 1.511~3.107) and FPG ≥ 7.00 mmol/L (OR: 2.779, 95% CI: 1.359~5.685) among overweight and obese adults, while AST levels did not correlate with FPG levels

Conclusions: The elevation of ALT levels was associated with the increased levels of FPG among overweight and obese adults in China, and ALT was a potential clinical bio-marker in diabetes risk assessment.

Keywords: Liver enzymes; Fasting plasma glucose; Adults

Strengths and limitations of this study

- The large sample of subjects was enrolled in our survey.
- To the best of our knowledge, this is the first study to explore the correlation between elevated liver enzymes and FPG among overweight and obese adults who were compared with a control group of adults with normal weight.
- The present study was designed as a cross-sectional study; therefore, direct causation cannot be concluded from the results.
- Supplementary information about γ-glutamyltransferase (GGT) levels and imaging studies was not collected; therefore, these factors could not be determined whether was associated with FPG among overweight and obese adults.

Introduction

Currently, diabetes is a major public health problem throughout the world. The International Diabetes Federation (IDF) estimated that approximately 382 million people suffered from diabetes around the world in 2013, and it was predicted to increase beyond 592 million in the next 25 years [1]. China, the largest developing country, has already been one of countries with a high incidence of diabetes. Recently a survey has revealed that more than one in ten of representative of the Chinese adults have diabetes [2]. Fasting plasma glucose (FPG) is the most commonly used indicator of diabetes. FPG monitoring is of significance in the prevention of diabetes.

The liver is the site of glycogen synthesis and glyconeogenesis, which plays an important role in maintaining the stable level of blood glucose in conjunction with the pancreas, muscle, adipose tissue and other organs [3-4]. Beyond that, the liver also regulates peripheral insulin sensitivity and participates in insulin degradation by secreting some molecules, such as selenoprotein P, angiopoietin-related growth factor [5-6]. Recent contributions have sought to clarify the relationship of the liver with type 2 diabetes [7-8]. Liver aminotransferases tests, the most frequent liver tests for evaluating the hepatocellular injury in clinic, involve alanine transaminase (ALT) and aspartate aminotransferase. (AST) that are found in the liver, serum as well as other organ tissues [9-11]. Several studies reported that the elevation of liver aminotransferases was indicative of insulin sensitivity reduction, insulin resistance, and the development of type 2 diabetes [12-14]. Related studies observed a significant association of ALT levels with the risk of type 2 diabetes [9, 14-15].

Researchers reported that body mass index (BMI) was a risk factor for changes of FPG levels [16], and liver aminotransferases levels [17]. Previous study found the association of ALT and AST levels with FPG levels was significant [3,14,18], however, those studies investigating the association between liver enzymes and FPG levels were conducted in the general population by considering BMI as a confounding factor. Until now, few studies have been performed to investigate the whether the association of liver enzymes levels with FPG levels varies in normal weight adults compared to overweight and obese adults.

On these grounds, the aim of this study was to determine the correlation between the FPG levels and liver enzymes elevation among overweight and obese adults who compared with a control group of

adults with normal weight in a cross-sectional study.

Materials and Methods

Study population

This cross-sectional study was conducted in 2014, in Guangdong Province, China. Initially, 3726 healthy inhabitants who underwent health examination (mean age: 60.32 years, ≥18 years old) were recruited in local Community Health Service Agencies At baseline examination, 574 participants with a history of diabetes, hepatitis B and all other liver diseases were excluded from the study; 141 participants with a BMI of less than 18.5Kg/m^2 were excluded. Further, participants with missing or invalid data on FPG levels and liver related indexes were also excluded, leaving a total of 2915 eligible participants (Figure 1). On the basis of data from this study, subjects were classified to two groups (normal weight, overweight and obesity) according to BMI (calculated as weight in kilograms divided by height meters squared). Individuals with a BMI between 18.5 and 23.9 Kg/m^2 were grouped as normal weight (n=1788), and those with a BMI of 24 Kg/m^2 or higher were grouped as overweight and obesity (n=1127). In the next step, we stratified overweight and obese adults into three groups according to their FPG levels: FPG < 5.56 mmol/L, 5.56 ≤ FPG <7.00 mmol/L and FPG ≥ 7.00 mmol/L. Each individual received written information about the aim of the study. If he/she decided to participate, a written informed consent was obtained.

Procedures

Data were collected via face-to-face interviews performed by either a physician or a nurse (the healthcare staff from local Community Health Service Agencies). The interviewers received training to improve their interview skills and standardize the procedures of data collection. All interviews took place in local Community Health Service Agencies, and the data were collected by using structured study questionnaires. After this investigation, the data were checked by the staff who have already received the training.

General examination

Information on participants' demographic characteristics (age, gender, marital status and education level), health-related characteristics (physical activity, smoking, drinking and BMI) and history of

diseases (diabetes, hepatitis B and all other liver diseases) was included by questionnaires. Marital status was categorized as "Single", "married", and "Divorce or Widowed". Education level was divided into four categories (including no school, primary school, middle school and high school or above). Physical activity was categorized as "every day", "more than once a week", "seldom", and "never". Smoking was categorized as "non-smoker", "smoker", and "ex-smoker". Drinking was divided into three categories, "regularly", "seldom", and "never". Smokers were defined as those who smoked one or more cigarettes per day for at least 6 months. Regular drinkers were defined as those who drank alcohol on average more than once a week within the last year.

Anthropometric parameters (height and weight) were collected in replicate and mean values were used in the study. FPG, albumin (ALB), direct bilirubin (DBIL), indirect bilirubin (IBIL), total bilirubin (TBIL), ALT and AST levels were measured in the local Community Health Service Agencies after an over 8 hours fasting.

Statistics analyses

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago,IL, USA). Data were presented as mean ± SD and frequencies (percentage). Continuous variables were analyzed by using one-way ANOVA, and categorical variables were compared by using the X^2 test. Partial correlation was performed to investigate the relationship between BMI and related indexes, to determine the correlation between FPG levels and liver tests among all participants. The levels of FPG in the baseline tertiles of AST and ALT levels were analyzed by using one-way ANOVA. The increasing risk of FPG levels on account of the elevation of liver enzymes was assessed by using multivariate logistic regression analysis.

Results

The present study included a total of 2915 adults comprising of 1788 (61.3%) individuals with normal weight and 1127 (38.7%) individuals with overweight and obesity. The Partial correlation coefficient between BMI and related indexes was shown in Table 1. All variables were significantly correlated with BMI, except for ALB, IBIL and TBIL. Furthermore, FPG and ALT levels correlated better with BMI than other indexes.

Notably, AST and ALT levels were not correlated with FPG levels among participants with normal weight, Surprisingly, except for DBIL, all liver tests were associated with FPG levels among participants with overweight and obesity (Table 2). Of the two liver enzymes, ALT levels had a higher correlation with FPG levels than AST levels. Changes of FPG levels depending on the baseline tertiles of AST and ALT levels among overweight and obese adults were shown in Figure 2. The significant difference between ALT levels and FPG levels was observed ($P < 0.05$), but this was not true for AST levels ($P > 0.05$).

Mean levels of age and BMI, and the frequency of gender, marital status, education level, physical activity, smoking and drinking among participants with overweight and obesity were presented in Table 3 (Since there was no association between liver enzymes and FPG in Table 2, the analysis of the association between general characteristics with FPG levels among participants with normal weight wasn't performed). Mean age was 60.01 ± 12.67, 63.34 ± 12.06, and 64.75 ± 13.88 in FPG < 5.56 mmol/L, 5.56 ≤ FPG < 7.00 mmol/L and FPG ≥ 7.00 mmol/L groups, respectively. Compared with the FPG < 5.56 mmol/L group, the 5.56 ≤ FPG < 7/00 mmol/L and FPG ≥ 7.00 mmol/L groups displayed significantly higher age ($P < 0.05$). Mean BMI was 26.37 ± 2.18, 26.65 ± 2.21, and 26.92 ± 2.59 in FPG < 5.56 mmol/L, 5.56 ≤ FPG < 7/00 mmol/L and FPG ≥ 7.00 mmol/L groups, respectively. Compared with the FPG < 5.56 mmol/L group, the FPG ≥ 7.00 mmol/L group displayed significantly higher BMI ($P < 0.05$), but this was not true for the 5.56 ≤ FPG < 7.00 mmol/L group ($P > 0.05$). There was significant difference in terms of smoking between FPG ≥ 7.00 mmol/L and FPG < 5.56 mmol/L groups ($P < 0.05$), but there was no significant difference between 5.56 ≤ FPG < 7.00 mmol/L and FPG < 5.56 mmol/L groups. In addition, compared with the FPG < 5.56 mmol/L group, the 5.56 ≤ FPG < 7.00 mmol/L and FPG ≥ 7.00 mmol/L groups did not display any significant difference in term of gender, marital status, education level, physical activity and drinking.

In a model adjusting for health-related factors and liver tests, multivariate logistic regression analysis confirmed a significant correlation between FPG levels and liver enzymes levels (Table 4). The highest tertiles of ALT levels remained significantly associated with FPG levels with an OR of 2.166 (95% CI: 1.511~3.107) in 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, and with an OR of 2.779 (95% CI: 1.359~5.685) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L, while AST levels did not correlate with FPG levels in 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L and FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L. Age showed an OR of 1.025 (95% CI: 1.013~1.036) in 5.56 ≤ FPG < 7.00

mmol/L vs. FPG < 5.56 mmol/L, and 1.035 (95% CI: 1.014–1.056) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L. However, ALB and IBIL levels displayed an OR of 0.955 (95% CI: 0.928–0.982) and 0.891 (95% CI: 0.806–0.985), respectively, in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L. BMI was not associated with FPG levels whether in 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, or FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L ($P > 0.05$).

Discussion

In the present study, AST and ALT levels were not related to FPG levels among adults with normal weight. Interestingly, ALT and AST were associated with FPG levels among overweight and obese participants (Table 2). The association of elevated liver enzymes concentrations with the increased risk of diabetes among overweight and obese adults may be partly explained by the obesity-related diabetes. Fall and his colleagues found that a causal effect of adiposity on ALT levels in a Mendelian randomization analysis study, and individuals with higher BMI had higher levels of ALT [17], which was consistent with findings of prior study [10]. A published meta-analysis study mentioned that elevated liver fat was a risk factor for the development of diabetes [9]. Mechanistically, increased intrahepatic fat content is bi-directionally associated with insulin resistance, which in its turn can lead to increased glucose output from the liver [9, 19-20].

In our study, changes of FPG levels were shown in Figure 2 depending on the baseline tertiles of AST and ALT levels among overweight and obese adults. An earlier study also reported that FPG levels increased with the elevation of AST and ALT levels among semiconductor workers who underwent three cycles of health check-ups [14]. A multicenter cross-sectional study reported that subjects in the highest ALT or AST group had higher FPG levels [21]. Qin et al reported that the cumulative incidence of impaired fasting glucose (defined as 5.6 mmol/L to 6.9 mmol/L) was significantly higher in the highest quartiles of liver enzymes than in the lowest quartiles [3]. Insulin resistance and insulin sensitivity reduction may be the key physiopathological mechanism of the elevation FPG levels [12-13]. An epidemiologic study conducted in 10800 middle-aged population noted that elevated liver enzymes levels were positively related to insulin resistance [22]. Evidence suggested that liver enzymes activities, even within the normal range, were strongly associated with both peripheral and hepatic insulin resistance, and can reduce hepatic insulin extraction among healthy men and women in a large cohort [13].

A univariate analysis was performed between general characteristics and FPG levels among overweight and obese adults (Table 3). The results showed that age, smoking and BMI were associated with FPG levels. In addition, the proportion of subjects who drank alcohol regularly and smoked was slightly small in the study population. The explanation as followed: 1) Most subjects (731, 64.9%) were female in our study; 2) The study subjects were overweight and obese adults, most of them may have gotten rid of some bad habits, such as smoking and drinking.

The present study, in agreement with previous reports [17, 23], demonstrated an association of elevated liver enzymes levels with FPG levels. The highest tertiles of ALT levels, but not AST levels, were significantly associated with FPG levels in this research (Table 4). In the identification of liver injury, the specificity of the ALT levels is better than the levels of AST [24]. Mainous et al analyzed a nationally representative sample of the noninstitutionalized US population, also confirming that ALT levels, but not AST levels, were independently linked with undiagnosed diabetes (FPG ≥ 126 mg/dl) as well as impaired fasting glucose.(100 ≤ FPG ≤ 125 mg/dl) [25]. Perera and his colleagues found that ALT and AST levels were associated with FPG levels in men, but only ALT levels were related to FPG levels in women [26]. Lu et al clarified that the effect of AST levels on higher diabetes risk might be due to ALT levels [27]. It may be because ALT is predominantly found in the liver, however, AST is not only found in the liver, but also in cardiac muscle, skeletal, brain and other organs.

In a previous study, ALT levels were associated with FPG levels in FPG ≥ 126 mg/dL vs. FPG < 100 mg /dL (OR: 1.16, 95% CI: 1.00~1.35) [28]. In current study, the highest tertiles of ALT levels were associated with more than a twofold increase of FPG levels (in 5.56 ≤ FPG < 7.00 mmol/L vs. FPG <5.56 mmol/L), independently of conventional risk factors. In depth, the highest tertiles of ALT levels were more significantly correlated with FPG levels in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L (Table 4), similar to a recent study [29]. Gonzálezpérez et al demonstrated that compared to normal ALT levels, the relative risk (RR) to the incidence of impaired fasting glucose (100 ≤ FPG ≤ 125 mg/dl) and diabetes (FPG ≥ 126 mg/dl) depending on the levels of ALT was 3.09 in borderline elevated ALT levels and 1.59 in elevated ALT levels [29]. The following mechanisms may be regarded as the causes of association between elevated liver enzymes levels and the increased risk of elevated FPG levels. 1) Elevated ALT levels reflected potential chronic inflammation and increased oxidative stress, which may impair insulin signaling in the liver and other organ tissues [13, 30]; 2) Elevated ALT levels could reflect life-long hepatitis virus infection, which can result in diabetes [31]; 3) The testosterone levels may be the mediator

between ALT levels and the risk of diabetes. Recent studies have revealed the role of low testosterone in diabetes [32], and that poor liver function may reduce testosterone production [33].

Our study was conducted in the Community Health Service Agencies, in Guangdong Province of China, and it may imply that the generalisability of our results is limited to this region. Additionally, participants with a history of diabetes, hepatitis B, all other liver diseases, and participants with a BMI of less than 18.5 Kg/m^2 were excluded from the study, so our results are not applicable to these subjects.

Limitations of the current study included the absence of γ-glutamyltransferase (GGT) levels and imaging studies. Recent literature reported that a moderate elevation of GGT levels within the normal range was a strong risk predictor for the onset of diabetes in a large non-obese population [34]. GGT may be a better predictor of diabetes than ALT [35]. However, in a meta-analysis of pooled population of 20 studies including 117020 patients followed-up for a median period of 5 years, NAFLD was associated with an increased risk of incident diabetes with a higher risk for ALT than GGT [36]. Recent studies noted that imaging studies will likely provide a new opportunity for investigating the association of the liver with diabetic disease [37-38]. In addition, our study design was cross-sectional and cannot provide insight into the development of diabetes over time. The strengths of this study include control of some important confounders such age, smoking and drinking. More importantly, this was the first time, to the best of our knowledge, to evaluate the correlation between FPG levels and the elevation of liver enzymes among overweight and obese adults who compared with a control group of adults with normal weight in China.

Conclusions

In summary, a strong association was observed between ALT levels and FPG levels among overweight and obese adults in China. The elevation of ALT levels should be considered as useful markers to identify individuals at the high risk of diabetes in China.

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Contributors: LLH, YPJ and PXW conducted the data analyses. LLH and DHG drafted the manuscript. DHG, HYX, STT and XXW finalized the manuscript with inputs from all authors. All authors contributed to the development of the study framework, interpretation of the results, revisions of successive drafts of the manuscript, and approved the version submitted for publication.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

Ethical approval: The protocol of this study was approved by the ethics committee of the Community Health Service Agencies of Liaobu town, Dongwan city, Guangdong province. The ethical code is 20130410.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Data sharing statement This database is first used in this study. The database belongs to our team, and if shared, you need to get their permission.

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Figure Legends

Fig 1 Flow chart in the selection of study population

Fig 2 Changes of fasting plasma glucose (FPG) levels depending on the baseline tertiles of aspartate aminotransferase (AST) and alanine transaminase (ALT) levels among overweight and obese adults

Table 1 Partial correlation between BMI and related indexes (n=2915)

Related indexes	Partial correlation coefficient (Controlling age, gender)	*p*
FPG	0.078	<0.001***
ALB	-0.023	0.214
DBIL	-0.047	0.010*
IBIL	-0.004	0.823
TBIL	-0.035	0.058
ALT	0.169	<0.001***
AST	0.045	0.014*

BMI: body mass index; FPG: fasting plasma glucose; ALB: albumin; DBIL: direct bilirubin; IBIL: indirect bilirubin ; TBIL: total bilirubin; ALT: alanine transaminase; AST: aspartate aminotransferase; * p <0.05; *** p <0.001.

Table 2 Partial correlation between FPG levels and liver tests among all participants (n=2915)

	Normal weight group	Overweight and obesity group

Liver tests	Partial correlation coefficient	*p*	Partial correlation coefficient	*p*
ALB	-0.057	0.015*	-0.097	0.001**
DBIL	-0.024	0.310	0.033	0.275
IBIL	-0.010	0.682	-0.111	<0.001***
TBIL	-0.035	0.137	-0.068	0.022*
ALT	0.013	0.573	0.078	0.008**
AST	-0.039	0.097	0.070	0.019*

Partial correlation coefficient: controlling age, gender and BMI; BMI: body mass index; FPG: fasting plasma glucose; ALB: albumin; DBIL: direct bilirubin; IBIL: indirect bilirubin； TBIL: total bilirubin; ALT: alanine transaminase; AST: aspartate aminotransferase; * p <0.05.

Table 3 General characteristics associated with FPG levels among participants with overweight and obesity (n=1127)

Variables	FPG < 5.56 (n=744)	5.56 ≤ FPG < 7.00 (n=310)	FPG ≥ 7.00 (n=73)
Age, years (m, SD)	60.01 ± 12.67	63.34 ± 12.06*	64.75 ± 13.88**
Gender (n, %)			
Male	255 (64.4)	106 (26.8)	35 (8.8)
Female	489 (66.9)	204 (27.9)	38 (5.2)
Marital status (n, %)			
Single	46 (73.0)	12 (19.0)	5 (7.9)
Married	635 (65.6)	275 (28.4)	58 (6.0)
Divorce or Widowed	36 (69.2)	11 (21.2)	5 (9.6)
Education level (n, %)			
No school	35 (62.5)	17 (30.4)	4 (7.1)
Primary school	126 (60.3)	69 (33.0)	14 (6.7)
Middle school	221 (65.6)	91 (27.0)	25 (7.4)

High school or above	43 (61.4)	23 (32.9)	4 (5.7)
Physical activity (n, %)			
Everyday	141 (60.0)	76 (32.3)	18 (7.7)
More than once a week	61 (68.5)	25 (28.1)	3 (3.4)
Seldom	30 (53.6)	19 (33.9)	7 (12.5)
Never	512 (68.5)	190 (25.4)	45 (6.0)
Smoking (n, %)			0.005**
Non-smoker	703 (66.5)	293 (27.7)	61 (5.8)
Smoker	33 (58.9)	15 (26.8)	8 (14.3)
Ex-smoker	8 (57.1)	2 (14.3)	4 (28.6)
Drinking (n, %)			
Regularly	5 (71.4)	1 (14.3)	1 (14.3)
Seldom	8 (44.4)	8 (44.4)	2 (11.1)
Never	731 (66.3)	301 (27.3)	70 (6.4)
BMI, Kg/m^2 (m, SD)	26.37 ± 2.18	26.65 ± 2.21	26.92 ± 2.59**

Data were presented as mean (SD) or n (%); FPG: fasting plasma glucose; BMI: body mass index; *P < 0.05 5.56 ≤ FPG < 7.00 mmol/L vs FPG < 5.56 mmol/L; **P < 0.05 FPG ≥ 7.00 mmol/L vs FPG < 5.56 mmol/L

Table 4. Odds ratios for FPG elevation by liver enzymes levels among participants with overweight and obesity (n=1127)

Variables	5.56 ≤ FPG < 7.00 VS FPG< 5.56		FPG≥ 7.00 VS FPG<5.56	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.025 (1.013~1.036)	<0.001***	1.035 (1.014~1.056)	0.001**
ALB	—		0.955 (0.928~0.982)	0.001**
IBIL	—		0.891 (0.806~0.985)	0.025*
ALT				
Q1	Reference			
Q2	1.358 (0.950~1.940)	0.093	1.893 (0.924~3.876)	0.81
Q3	2.166 (1.511~3.107)	<0.001***	2.779 (1.359~5.685)	0.005**

Statistical analysis by multivariate logistic regression (adjusted for age, smoking, BMI (body mass index) and liver tests); OR: odds ratio; CI: confidence interval; FPG: fasting plasma glucose; ALB: albumin; IBIL: indirect bilirubin; ALT: alanine transaminase.

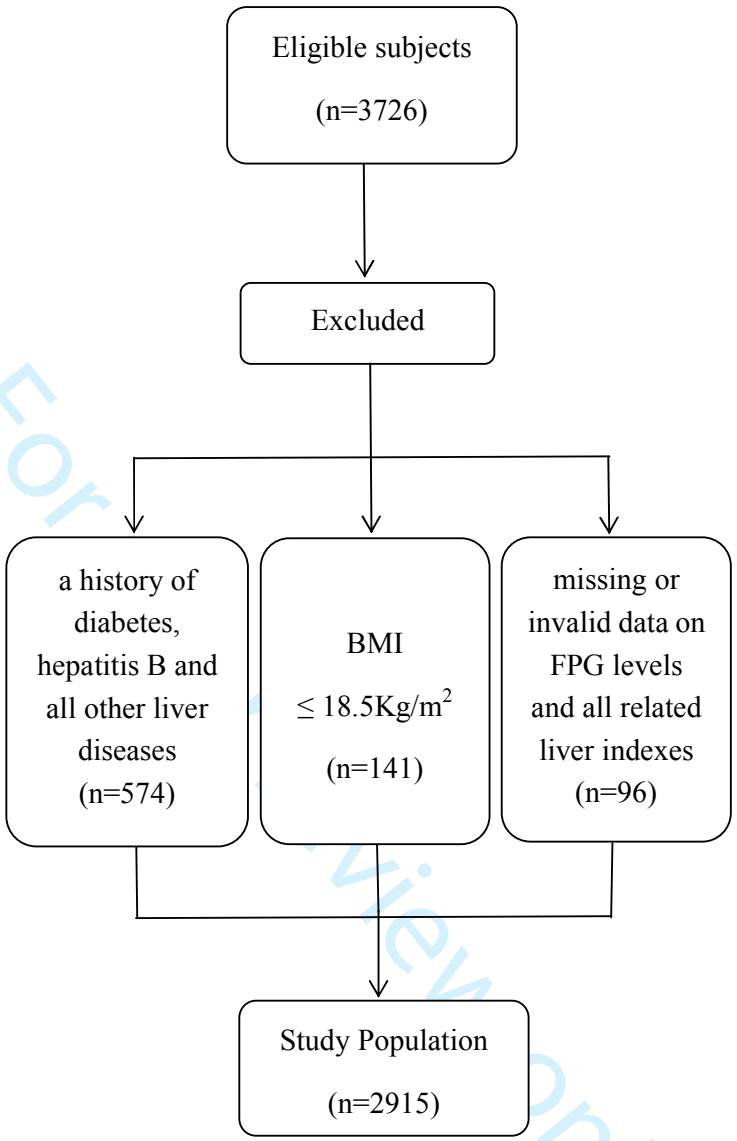

Fig 1

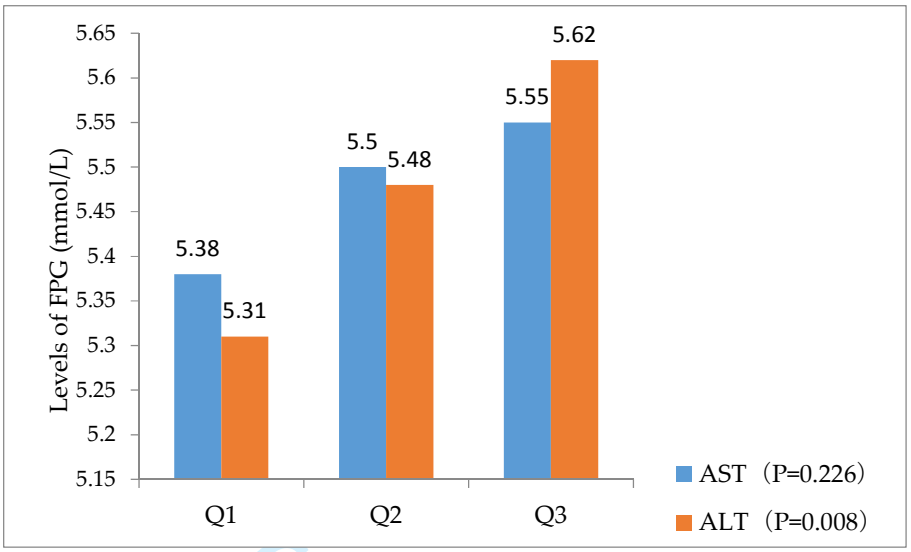

Fig 2

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 4)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 5)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants (Page 5)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 5-6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 5-6)
Bias	9	Describe any efforts to address potential sources of bias (Page 5)
Study size	10	Explain how the study size was arrived at (Page 5)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page 5)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 6)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 6)
		(*c*) Explain how missing data were addressed (Page 5)
		(*d*) If applicable, describe analytical methods taking account of sampling strategy (not applicable)
		(*e*) Describe any sensitivity analyses (Page 6)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Page 6, Table 1-3)
		(b) Give reasons for non-participation at each stage (not applicable)
		(c) Consider use of a flow diagram (Page 5)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 6-7, Table 1-3)
		(b) Indicate number of participants with missing data for each variable of interest (Not applicable)
Outcome data	15*	Report numbers of outcome events or summary measures (Page 7-8, Table 3-4)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Page 7-8, Table 4)

		(*b*) Report category boundaries when continuous variables were categorized (Page 5)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (Not applicable)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Not applicable)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 8-9)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 10)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 8-9)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 10)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 11)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association of liver enzymes levels with fasting plasma glucose levels in Southern China: a cross-sectional study

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Association of liver enzymes levels with fasting plasma glucose levels in Southern China: a cross-sectional study

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Abstract

Objective: Several studies have reported that liver enzymes levels were associated with fasting plasma glucose (FPG) levels. However, the association, stratified by body mass index (BMI) among people without diagnosed diabetes, remains to be elucidated, especially in Southern China. Therefore, our aim was to investigate the correlation between liver enzymes levels and FPG levels stratified by BMI among people who had not been diagnosed with diabetes before this study, in Southern China.

Design: Cross-sectional study

Participants and setting: 3056 individuals underwent real-time interviews and blood tests in Southern China. Participants were divided into three groups (underweight, normal weight, overweight and obesity) along a BMI cut-off.

Main outcome measured: Partial correlation was performed to investigate the relationship between FPG levels and liver tests. Multivariate logistic regression analyses were applied to calculate adjusted ORs for FPG levels associated with liver enzymes levels.

Results: There was no association between liver enzymes levels and FPG levels whether in underweight group or in normal weight group, but the significant correlation was observed in overweight and obesity group (alanine transaminase（ALT）, $P < 0.01$, aspartate aminotransferase(AST), $P < 0.05$). After adjusting for confounding factors, the highest tertiles of ALT still remained significantly positively related to FPG levels in overweight and obesity group, with an OR of 2.166 (95% CI: 1.511~3.107) in $5.56 \leq$ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, and with an OR of 2.779 (95% CI: 1.359~5.685) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L, but this was not the same for AST.

Conclusions: The association of liver enzymes levels with FPG levels differed along a BMI cut-off. ALT levels were significantly positively associated with FPG levels in overweight and obesity group, but not in other two groups; AST levels was not associated with FPG levels in all groups.

Keywords: Liver enzymes; Fasting plasma glucose; Southern China; Cross-sectional study

Strengths and limitations of this study

- A large sample of subjects were enrolled in our survey.
- To the best of our knowledge, this is the first study to explore the correlation between liver enzymes levels and FPG levels stratified by BMI among people who had not been diagnosed with diabetes before this survey, in Southern China.
- The present study was designed as a cross-sectional study; therefore, direct causation cannot be concluded from the results.
- Supplementary information about γ-glutamyltransferase (GGT) levels, imaging studies, cholesterol, triglycerides was not collected; therefore, these factors could not be determined whether was associated with FPG, some factors such as cholesterol, triglycerides levels, could not be included in the adjustments of our multivariate logistic regression analyses.

Introduction

Diabetes, one of prevalent chronic diseases, has emerged as a major public health issue owing to its increased prevalence in many countries, affecting approximately 382 million people throughout the world [1]. China, the largest developing country, is also no exception to suffering from the high incidence of diabetes. Recently, a survey has revealed that more than one in ten of Chinese adults were exposed to diabetes [2]. Therefore, early identification of individuals at high risk of diabetes is essential for decreasing the prevalence of diabetes.

The liver is the site of glycogen synthesis and glyconeogenesis, which plays an important role in maintaining the stable level of blood glucose in conjunction with the pancreas, muscle, adipose tissue and other organs [3-4]. Liver enzymes, the most frequent liver tests for evaluating the liver function in clinic, involve alanine transaminase (ALT) and aspartate aminotransferase. (AST) [5-7]. Related studies suggested that the elevation of liver enzymes levels was indicative of insulin sensitivity reduction, insulin resistance, and the development of type 2 diabetes [8-10]. Fasting plasma glucose (FPG) level is the most commonly used index to monitor the occurrence of early type 2 diabetes. which is of great significance in the prevention of diabetes. Although previous study have reported that liver enzymes levels were significantly associated with FPG levels [3,10-11], the evidence was still insufficient, because the results reported are inconsistent according to the populations studied, such as the population in different regions, the population with different body mass index (BMI). In addition, as we know, almost all related studies simply regarded BMI as an adjustment variable to investigate the relationship between liver enzymes levels and FPG levels in general population. Therefore, the aim of this study was to determine the association of liver enzymes levels with FPG levels, stratified by BMI, among people who had not been diagnosed with diabetes before this survey, in Southern China. If the elevated liver enzymes levels were significantly associated with the elevation of FPG levels, it may have implication in considering liver enzymes as effective molecular markers for the early detection of diabetes high-risk individuals with different BMI cut-points, and health policy makers can develop targeted interventions to prevent the early occurrence of type 2 diabetes among people with different BMI cut-points.

Materials and Methods

Study population

This cross-sectional study was conducted in Guangdong Province of China in 2014. Initially, 3726 healthy inhabitants who underwent a general health examination (mean age: 60.32 years, ≥18 years old) were recruited in local Community Health Service Agencies. The health examination included recording of general characteristics, medical history, anthropometric parameters and laboratory tests. 574 participants with a history of diabetes, hepatitis B and all other liver diseases were excluded from the study. Further, participants with missing or invalid data on FPG levels or liver related indexes were also excluded, leaving a total of 3056 eligible participants (Figure 1). On the basis of data from this study, subjects were classified to three groups (underweight, normal weight, overweight and obesity) along a BMI cut-off (BMI was calculated as weight in kilograms divided by height meters squared). Individuals with a BMI <18.5, 18.5 ~23.9 Kg/m^2 ,≥24 Kg/m^2 were divided into underweight group (n=141), normal weight group (n=1788), and overweight and obesity group (n=1127), respectively. Then, we stratified overweight and obese adults into three groups according to FPG levels: FPG < 5.56 mmol/L, 5.56 ≤ FPG <7.00 mmol/L and FPG ≥ 7.00 mmol/L. Each individual received written information about the aim of the study. If he/she decided to participate, a written informed consent was obtained.

General characteristics

Information on participants' demographic characteristics (age, gender, marital status and education level), health-related characteristics (physical activity, smoking, drinking and BMI) and medical history (diabetes, hepatitis B and all other liver diseases) was included in questionnaires. Marital status was categorized as "Single", "married", and "Divorce or Widowed". Education level was divided into four categories (no school, primary school, middle school and high school or above). Physical activity was categorized as "every day", "more than once a week", "seldom", and "never". Smoking was grouped as "non-smoker", "smoker", and "ex-smoker". Drinking was divided into three categories, "regularly", "seldom", and "never". Smokers were defined as those who smoked one or more cigarettes per day for at least 6 months. Regular drinkers were defined as those who drank alcohol on average more than once a week within the last year.

Anthropometric parameters and laboratory tests

Anthropometric parameters (height and weight) were measured by trained staff, following a standardized protocol. The data were collected in replicate and mean values were calculated in the study. After an overnight fast (at least 8 hours), venous blood samples from participants were obtained and analysed by PPI automatic biochemical analyzer (Roche Company, Germany) for FPG, albumin (ALB), direct bilirubin (DBIL), indirect bilirubin (IBIL), total bilirubin (TBIL), ALT and AST levels.

Tertiles of erythrocyte parameters levels

Liver enzymes levels were categorized into tertiles on the basis of individual distributions in overweigh and obesity group. ALT: Q1 <17 U/L, Q2=17~25 U/L, Q3 ⩾25 U/L; AST: Q1 <20 U/L, Q2=20~24 U/L, Q3 ⩾24 U/L.**Procedures**All data were collected on the same day via face-to-face interviews and blood tests performed by either a physician or a nurse (the healthcare staff from local Community Health Service Agencies). The interviewers received training to improve their interview skills and standardize the procedures of data collection. Besides, several supervisors were arranged to verify the authenticity of the data.

Patient and public involvement

The role of study subjects in our survey was participants. They were not involved in the development of the research question and outcome measures, the recruitment of subjects and the conduct of the study. After completing this survey, we sent each participant a letter describing detailed results of this study.

Statistics analyses

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago,IL, USA). Data were presented as mean ± SD and frequencies (percentage). A one-way ANOVA was conducted to evaluate differences in age and BMI according to FPG levels, to test mean levels of FPG dependent on the tertiles for ALT and AST in overweight and obesity group. The X^2 test was used to compare the frequency of general characteristics (categorical variables) according to FPG levels in overweight and obesity group. Partial correlation was performed to investigate the relationship between BMI and related indexes among all participants shown in Table 1, to determine the correlation between FPG levels and liver tests stratified by BMI.. Multivariate

logistic regression analyses were applied to calculate the adjusted ORs for FPG levels associated with liver enzymes levels in overweight and obesity group. (Since there was no association between liver enzymes and FPG in underweight group and normal group, respectively shown in Table 2, the one-way ANOVA, X^2 test and m ultivariate logistic regression were not performed in these two groups.).

Results

The Partial Correlation and One-Way ANOVA

A total of 3056 adults were included in this study, comprising of 141 (4.6%) individuals with underweight, 1788 (58.5%) individuals with normal weight and 1127 (36.9%) individuals with overweight and obesity. The partial correlation coefficient between BMI and related indexes was shown in Table 1. Every index was significantly correlated with BMI, except for ALB and IBIL. Compared with other indexes, FPG and ALT were more correlated with BMI.

Further, the partial correlation was conducted to explore the relationship between FPG levels and liver tests stratified by BMI (shown in Table 2). Notably, AST and ALT levels were not associated with FPG levels in underweight group and normal weight group, respectively, but the significant association of AST and ALT levels with FPG levels was observed in overweight and obesity group. Of the two liver enzymes, ALT (r =0.097, $P < 0.05$) levels had a stronger correlation with FPG levels than AST levels (r =0.070, $P < 0.05$). Mean levels of FPG depending on the baseline tertiles of AST and ALT levels among overweight and obese adults were shown in Figure 2. FPG levels were positively related to ALT levels ($P < 0.05$), but not to AST levels ($P > 0.05$).

Association of general characteristics with FPG

Mean levels of age and BMI, and the frequency of gender, marital status, education level, physical activity, smoking and drinking according to FPG levels, in overweight and obesity group were presented in Table 3 Mean age was 60.01 ± 12.67, 63.34 ± 12.06, and 64.75 ± 13.88 in FPG < 5.56 mmol/L, 5.56 ≤ FPG < 7.00 mmol/L and FPG ≥ 7.00 mmol/L groups, respectively. Compared with the FPG < 5.56 mmol/L group, the 5.56 ≤ FPG < 7/00 mmol/L and FPG ≥ 7.00 mmol/L groups displayed significantly higher age ($P < 0.05$). Mean BMI was 26.37 ± 2.18, 26.65 ± 2.21, and 26.92 ± 2.59 in FPG < 5.56 mmol/L, 5.56 ≤ FPG < 7/00 mmol/L and FPG ≥ 7.00 mmol/L groups, respectively. Compared with the FPG < 5.56 mmol/L group, the FPG ≥ 7.00 mmol/L group displayed significantly

higher BMI ($P < 0.05$), but this was not true for the $5.56 \leq$ FPG < 7.00 mmol/L group ($P > 0.05$). In terms of smoking, there was a significant difference between FPG ≥ 7.00 mmol/L and FPG < 5.56 mmol/L groups ($P < 0.05$), but not between $5.56 \leq$ FPG < 7.00 mmol/L and FPG < 5.56 mmol/L groups.

Multivariate logistic regression analysis model

Adjusted ORs for FPG levels associated with liver enzymes levels in overweight and obesity group were listed in Table 4. Additionally, only those variables that were significantly correlated with FPG levels were presented in Table 4. After adjusting for potential confounders (age, smoking, BMI and liver tests), the highest tertiles of ALT levels remained significantly positively correlated with FPG levels with an OR of 2.166 (95% CI: 1.511~3.107) in $5.56 \leq$ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, and with an OR of 2.779 (95% CI: 1.359~5.685) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L, but this was not the same for AST.

Age showed an OR of 1.025 (95% CI: 1.013~1.036) in $5.56 \leq$ FPG < 7.00 mmol/L vs. FPG $<$ 5.56 mmol/L, and 1.035 (95% CI: 1.014–1.056) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L. However, ALB and IBIL levels displayed an OR of 0.955 (95% CI: 0.928–0.982) and 0.891 (95% CI: 0.806–0.985), respectively, in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L.

Discussion

Partial correlation showed that AST and ALT levels were not associated with FPG levels in underweight group and normal weight group, but the significantly positive association of AST and ALT levels with FPG levels was observed in overweight and obesity group (Table 2). This positive association among overweight and obese adults may be partly explained by the obesity-related diabetes. For example, Fall and his colleagues found that a causal effect of adiposity on ALT levels in a Mendelian randomization analysis study, and individuals with higher BMI had higher ALT levels [12], which was consistent with findings of prior study [6]; a published meta-analysis study mentioned that elevated liver fat was a risk factor for the development of diabetes [5]. Mechanistically, increased intrahepatic fat content is bi-directionally associated with insulin resistance, which in its turn can lead to increased glucose output from the liver [5, 13-14].

An earlier study has demonstrated that FPG levels increased with the elevation of AST and ALT levels among semiconductor workers who underwent three cycles of health check-ups, indicating that liver enzymes are potential markers for early detection of diabetes [10]. In our study, mean levels of FPG were shown in Figure 2 depending on the baseline tertiles of AST and ALT levels in overweight and obesity group. Our results revealed that the elevated FPG levels were related to the increased levels of liver enzymes, similar to a recent study [15]. Additionally, Qin et al reported that the cumulative incidence of impaired fasting glucose (defined as 5.6 mmol/L to 6.9 mmol/L) was significantly higher in the highest quartiles of liver enzymes than that in the lowest quartiles [3]. Insulin resistance and insulin sensitivity reduction may be the key physiopathological mechanism of this positive association between liver enzymes levels and FPG levels [8-9]. An epidemiologic study conducted in 10800 middle-aged populations noted that elevated liver enzymes levels were closely related to insulin resistance [16]. Evidence of a large cohort suggested that liver enzymes activities, even within the normal range, were strongly associated with both peripheral and hepatic insulin resistance, and can reduce hepatic insulin extraction among healthy men and women [9]. It has been found that insulin resistance and insulin sensitivity reduction can lead to increased glucose output [5, 13-14].

In the identification of liver injury, ALT is more specific than AST [17]. For instance, Perera and his colleagues found that both ALT and AST levels were associated with FPG levels in men, but only ALT levels were related to FPG levels in women [18]; Mainous et al, analyzing a nationally representative sample of the noninstitutionalized US population, found that ALT levels, but not AST levels, were independently linked with undiagnosed diabetes (defined as FPG $\geq$ 126 mg/dl) as well as impaired fasting glucose.(defined as 100 $\leq$ FPG $\leq$ 125 mg/dl) [19], consistent with our results in general. In our study, after adjusting for potential confounders (age, smoking, BMI and liver tests), ALT levels remained significantly positively correlated with FPG levels both in 5.56 $\leq$ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/Land FPG $\geq$ 7.00 mmol/L vs. FPG < 5.56 mmol/L, but this was not the same for AST. It may be that ALT predominantly exists in liver, however, not only is AST found in the liver, but in cardiac muscle, skeletal, brain and other organs. Lu et al clarified that the effect of AST levels on diabetes risk was partly due to ALT levels 20].

In a previous research, ALT levels were associated with FPG levels in FPG $\geq$ 126 mg/dL vs. FPG < 100 mg /dL (OR: 1.16, 95% CI: 1.00~1.35) [21]. In the present study, the highest tertiles of ALT levels were associated with more than a twofold increase of FPG levels (in 5.56 $\leq$ FPG < 7.00 mmol/L

vs. FPG <5.56 mmol/L), independently of conventional risk factors. In depth, the highest tertiles of ALT levels were more significantly correlated with FPG levels in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L (Table 4), similar to an early study [22]. Gonzálezpérez et al reported that compared to normal ALT levels, the relative risk (RR) for the incidence of impaired fasting glucose (100 ≤ FPG ≤ 125 mg/dl) and diabetes (FPG ≥ 126 mg/dl) depending on the levels of ALT was 3.09 in borderline elevated ALT levels and 1.59 in elevated ALT levels [22]. The following mechanisms may be regarded as the causes of the association between elevated liver enzymes levels and the increased risk of elevated FPG levels. 1) Elevated ALT levels reflected potential chronic inflammation and increased oxidative stress, which may impair insulin signaling in the liver and other organ tissues [9, 23]; 2) Elevated ALT levels could reflect life-long hepatitis virus infection, which can result in diabetes [24]; 3) The testosterone levels may be the mediator between ALT levels and the risk of diabetes. researchers have revealed the role of low testosterone in diabetes [25], and that poor liver function may reduce testosterone production [26].

Our study was conducted in the Community Health Service Agencies, in Guangdong Province of China, and it may imply that the generalisability of our results is limited to this region. Additionally, participants with a history of diabetes, hepatitis B, all other liver diseases were excluded from the study, so our results are not applicable to these subjects.

Limitations of the current study included the absence of γ-glutamyltransferase (GGT) levels and imaging studies. Recent literature reported that a moderate elevation of GGT levels within the normal range was a strong risk predictor for the onset of diabetes in a large non-obese population [27]. GGT may be a better predictor of diabetes than ALT [28]. Recent studies noted that imaging studies will likely provide a new opportunity for investigating the association of the liver with diabetic disease [29-30]. Then, supplementary information about the blood lipid, diseases types and medication history of subjects was not collected. Hence, some factors such as cholesterol, triglycerides levels, could not be included in the adjustments of our multivariate logistic regression analyses. In addition, our study design was cross-sectional, and direct causation cannot be concluded from the results.

Conclusions

The association of liver enzymes levels with FPG levels differed along a BMI cut-off. ALT levels were significantly positively associated with FPG levels in overweight and obesity group, but not in

underweight group and normal weight group; AST levels was not associated with FPG levels in all groups. This has important clinical implications for health makers. Liver enzymes may serve as effective indices for the early detection of diabetes high-risk individuals on a BMI dependent basis.

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Contributors: LLH, YPJ and PXW conducted the data analyses. LLH and DHG drafted the manuscript. DHG, HYX, STT and XXW finalized the manuscript with inputs from all authors. All authors contributed to the development of the study framework, interpretation of the results, revisions of successive drafts of the manuscript, and approved the version submitted for publication.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

Ethical approval: The protocol of this study was approved by the ethics committee of the Community Health Service Agencies of Liaobu town, Dongwan city, Guangdong province. The ethical code is 20130410.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Data sharing statement This database is first used in this study. The database belongs to our team, and if shared, you need to get their permission.

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Figure Legends

Fig 1 Flow chart in the selection of study population

Fig 2 Mean levels of fasting plasma glucose (FPG) levels depending on the baseline tertiles of aspartate aminotransferase (AST) and alanine transaminase (ALT) levels in overweight and obesity group

Table 1 Partial correlation between BMI and related indexes (n=3056)

Related indexes	Partial correlation coefficient (Controlling age, gender)	*p*
FPG	0.077	<0.001***
ALB	-0.010	0.573
DBIL	-0.049	0.008**
IBIL	-0.004	0.833
TBIL	-0.038	0.035*
ALT	0.165	<0.001***
AST	0.037	0.040*

BMI: body mass index; FPG: fasting plasma glucose; ALB: albumin; DBIL: direct bilirubin; IBIL: indirect bilirubin ; TBIL: total bilirubin; ALT: alanine transaminase; AST: aspartate aminotransferase; * p <0.05, **p <0.01, *** p <0.001.

Table 2 Partial correlation between FPG levels and liver tests stratified by BMI (n=3056)

Liver tests	Underweight group (correlation coefficient, n=141)	Normal weight group (correlation coefficient, n=1788)	Overweight and obesity group (correlation coefficient, n=1127)
ALB	-0.042	-0.057*	-0.097**
DBIL	0.021	-0.024	0.033
IBIL	-0.005	-0.010	-0.111***
TBIL	-0.025	-0.035	-0.068*

ALT	0.011	0.013	0.078**
AST	-0.034	-0.039	0.070*

Partial correlation coefficient: controlling age, gender and BMI; BMI: body mass index; FPG: fasting plasma glucose; ALB: albumin; DBIL: direct bilirubin; IBIL: indirect bilirubin； TBIL: total bilirubin; ALT: alanine transaminase; AST: aspartate aminotransferase; * p <0.05, **p <0.01, *** p <0.001.

Table 3 General characteristics associated with FPG levels in overweight and obesity group (n=1127)

Variables	FPG < 5.56 (n=744)	5.56 ≤ FPG < 7.00 (n=310)	FPG ≥ 7.00 (n=73)
Age, years (m, SD)	60.01 ± 12.67	63.34 ± 12.06*	64.75 ± 13.88**
Gender (n, %)			
Male	255 (34.4)	106 (34.2)	35 (47.9)
Female	489 (65.7)	204 (65.8)	38 (52.1)
Marital status (n, %)			
Single	46 (6.4)	12 (4.0)	5 (7.4)
Married	635 (88.6)	275 (92.3)	58 (85.3)
Divorce or Widowed	36 (5.0)	11 (3.7)	5 (7.4)
Education level (n, %)			
No school	35 (8.2)	17 (8.5)	4 (8.5)
Primary school	126 (29.6)	69 (34.5)	14 (29.8)
Middle school	221 (52.0)	91 (45.5)	25 (53.2)
High school or above	43 (10.1)	23 (11.5)	4 (8.5)
Physical activity (n, %)			
Everyday	141 (19.0)	76 (24.5)	18 (24.7)
More than once a week	61 (8.2)	25 (8.1)	3 (4.1)
Seldom	30 (4.0)	19 (6.1)	7 (9.6)

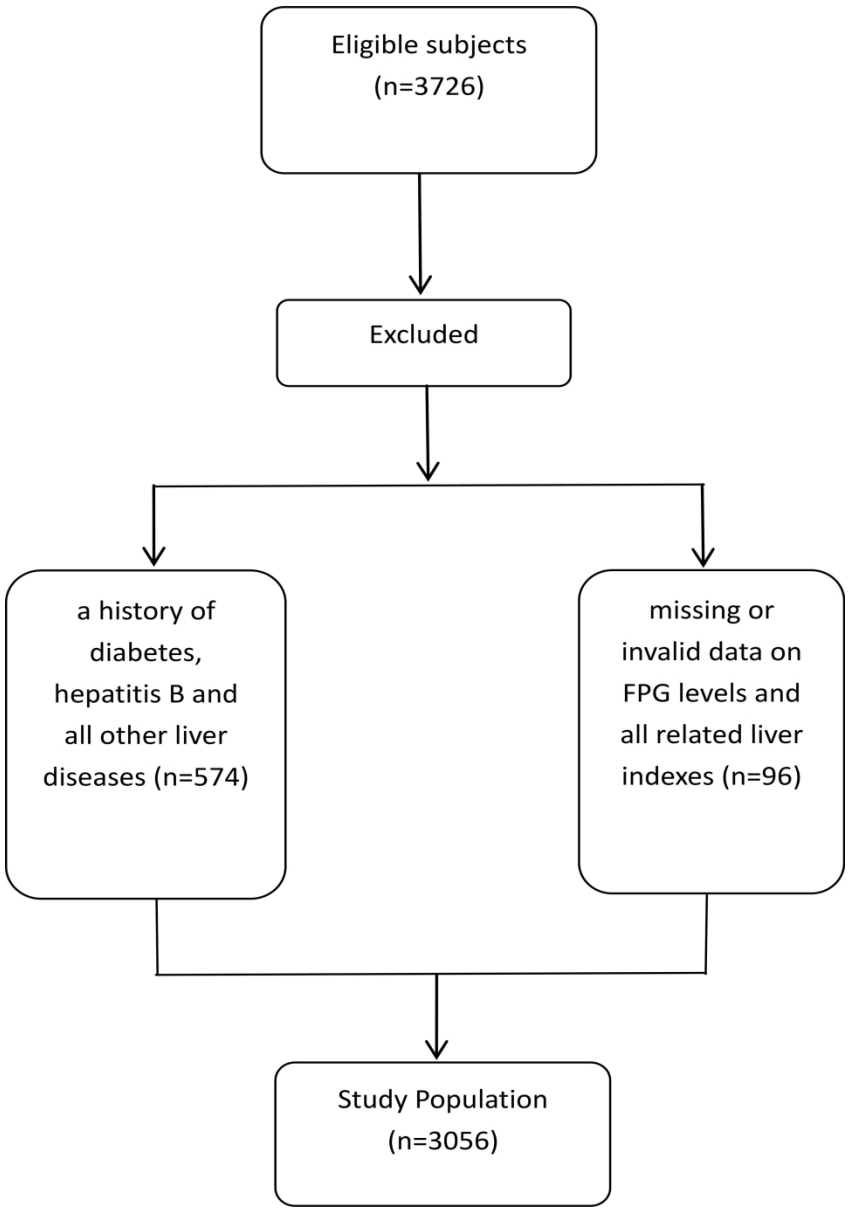
Never	512 (68.8)	190 (61.3)	45 (61.6)
Smoking (n, %)			0.005**
Non-smoker	703 (94.5)	293 (94.5)	61 (83.6)
Smoker	33 (4.4)	15 (4.8)	8 (11.0)
Ex-smoker	8 (1.1)	2 (0.6)	4 (5.5)
Drinking (n, %)			
Regularly	5 (0.7)	1 (0.3)	1 (1.4)
Seldom	8 (1.1)	8 (2.6)	2 (2.7)
Never	731 (98.3)	301 (97.1)	70 (95.9)
BMI, Kg/m^2 (m, SD)	26.37 ± 2.18	26.65 ± 2.21	26.92 ± 2.59**

Data were presented as mean (SD) or n (%); FPG: fasting plasma glucose; BMI: body mass index; *P < 0.05 5.56 ≤ FPG < 7.00 mmol/L vs FPG < 5.56 mmol/L; **P < 0.05 FPG ≥ 7.00 mmol/L vs FPG < 5.56 mmol/L

Table 4. Odds ratios for FPG elevation by liver enzymes levels in overweight and obesity group (n=1127)

Variables	5.56 ≤ FPG < 7.00 VS FPG< 5.56		FPG≥ 7.00 VS FPG<5.56	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.025 (1.013~1.036)	<0.001***	1.035 (1.014~1.056)	0.001**
ALB	—		0.955 (0.928~0.982)	0.001**
IBIL	—		0.891 (0.806~0.985)	0.025*
ALT				
Q1	Reference			
Q2	1.358 (0.950~1.940)	0.093	1.893 (0.924~3.876)	0.81
Q3	2.166 (1.511~3.107)	<0.001***	2.779 (1.359~5.685)	0.005**

Statistical analysis by multivariate logistic regression (adjusted for age, smoking, BMI (body mass index) and liver tests); OR: odds ratio; CI: confidence interval; FPG: fasting plasma glucose; ALB: albumin; IBIL: indirect bilirubin; ALT: alanine transaminase.



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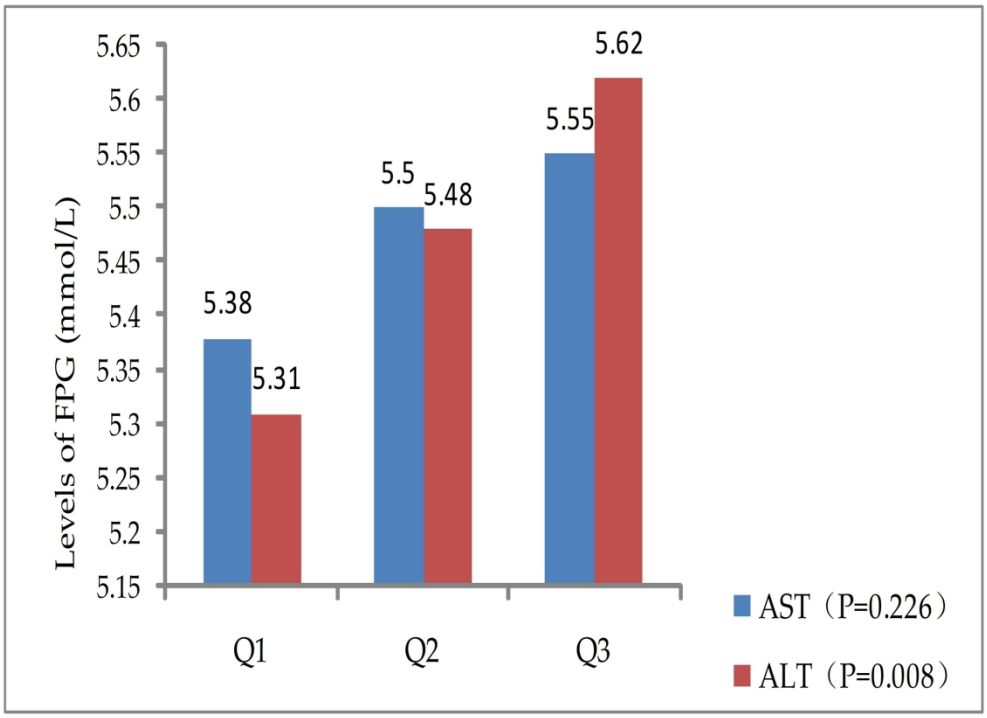

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 4)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 5)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants (Page 5)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 5-6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 5-6)
Bias	9	Describe any efforts to address potential sources of bias (Page 6, 8)
Study size	10	Explain how the study size was arrived at (Page 5)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page 5, 6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 6-7)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 6-7)
		(*c*) Explain how missing data were addressed (Page 5)
		(*d*) If applicable, describe analytical methods taking account of sampling strategy (not applicable)
		(*e*) Describe any sensitivity analyses (Page 6-7)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Page 7, Table 1-3)
		(b) Give reasons for non-participation at each stage (not applicable)
		(c) Consider use of a flow diagram (Figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 7-8, Table 1-3)
		(b) Indicate number of participants with missing data for each variable of interest (Not applicable)
Outcome data	15*	Report numbers of outcome events or summary measures (Page 7-8, Table 3-4)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Page 7-8, Table 4)

		(*b*) Report category boundaries when continuous variables were categorized (Page 5)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (Not applicable)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Not applicable)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 8-10)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 10)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 8-10)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 10)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 11)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association of liver enzymes levels with fasting plasma glucose levels in Southern China: a cross-sectional study

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Association of liver enzymes levels with fasting plasma glucose levels in Southern China: a cross-sectional study

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Abstract

Objective: According to several studies, liver enzymes levels were associated with fasting plasma glucose (FPG) levels. However, the association remains to be elucidated stratified by body mass index (BMI), especially in Southern China. Therefore, the aim of this study was to investigate the correlation between liver enzymes levels and FPG levels stratified by BMI in Southern China.

Design: Cross-sectional study

Participants and setting: 3056 individuals were involved in real-time interviews and blood tests in Southern China. Participants were divided into three groups (underweight, normal weight, overweight and obesity) along a BMI cut-off.

Main outcome measured: Partial correlation was performed to investigate the relationship between FPG levels and liver tests. Multivariate logistic regression analyses were applied to calculate adjusted ORs for FPG levels associated with liver enzymes levels.

Results: There was no association between liver enzymes levels and FPG levels either in underweight group or in normal weight group, however, the significant correlation was observed in overweight and obesity group (alanine transaminase(ALT), $P < 0.01$, aspartate aminotransferase(AST), $P < 0.05$). After adjusting for confounding factors, the highest tertiles of ALT still remained significantly positively related to FPG levels in overweight and obesity group, with an OR of 2.205 (95% CI: 1.442~3.371) in $5.56 \leq$ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, and with an OR of 2.297 (95% CI: 1.017~5.187) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L, but this was not ture for AST.

Conclusions: The association of liver enzymes levels with FPG levels differed along a BMI cut-off. ALT levels were significantly positively associated with FPG levels in overweight and obesity group, but not in other two groups; AST levels was not associated with FPG levels in all groups.

Keywords: Liver enzymes; Fasting plasma glucose; Southern China; Cross-sectional study

Strengths and limitations of this study

- A large sample of subjects was enrolled in this survey.
- To the best of our knowledge, this is the first study to explore the correlation between liver enzymes levels and FPG levels stratified by BMI among people who had not been diagnosed with diabetes before this survey, in Southern China.
- The present study was designed as a cross-sectional study; therefore, direct causation cannot be concluded from the results.
- Supplementary information about γ-glutamyltransferase (GGT) levels, imaging studies, cholesterol, triglycerides, was not collected; therefore, these factors could not be determined whether was associated with FPG, some factors such as cholesterol, triglycerides levels, could not be included in the adjustments of the multivariate logistic regression analyses.

Introduction

Diabetes, one of prevalent chronic diseases, has emerged as a major public health issue owing to its increased prevalence in many countries, affecting approximately 382 million people throughout the world [1]. China, the largest developing country, is also no exception to suffering from the high incidence of diabetes. Recently, a survey has revealed that more than one in ten of Chinese adults were exposed to diabetes [2]. Therefore, early identification of individuals at high risk of diabetes is essential for decreasing the prevalence of diabetes.

The liver is the site of glycogen synthesis and glyconeogenesis, which plays an important role in maintaining the stable level of blood glucose in conjunction with the pancreas, muscle, adipose tissue and other organs [3-4]. Liver enzymes, the most frequent liver tests for evaluating the liver function in clinic, include alanine transaminase (ALT) and aspartate aminotransferase. (AST) [5-7]. Related studies suggested that the elevation of liver enzymes levels was indicative of insulin sensitivity reduction, insulin resistance, and type 2 diabetes development [8-10]. Fasting plasma glucose (FPG) is the most commonly used index to monitor the occurrence of early type 2 diabetes. which is of great significance in the prevention of diabetes. Although previous studies have reported that liver enzymes levels were significantly associated with FPG levels [3,10-11], the evidence was still insufficient, because the results reported are inconsistent according to the population studied, such as the population in different regions, the population with different body mass index (BMI). In addition, as we know, almost all related studies simply regarded BMI as an adjustment variable to investigate the relationship between liver enzymes levels and FPG levels in general population, and few studies were conducted in Southern China. Therefore, the aim of this study was to determine the association of liver enzymes levels with FPG levels, stratified by BMI, among people who had not been diagnosed with diabetes before this survey, in Southern China. If the elevated liver enzymes levels were significantly associated with the elevation of FPG levels, it may have implication in considering liver enzymes as effective molecular markers for the early detection of diabetes high-risk individuals with different BMI cut-points, and health policy makers can develop targeted interventions to prevent the early occurrence of type 2 diabetes according to different BMI cut-points.

Materials and Methods

Study population

This cross-sectional study was conducted in Guangdong Province of China in 2014. Initially, 3726 healthy inhabitants who underwent a general health examination (mean age: 60.32 years, ≥18 years old) were recruited in local Community Health Service Agencies. All participants completed the survey, and the overall response rate was 100%.The health examination included recording of general characteristics, medical history, anthropometric parameters and laboratory tests. After excluded subjects (n=574) with a history of diabetes, hepatitis B and all other liver diseases, subjects (n=96) with missing or invalid data on FPG levels or liver related indexes, 3056 eligible participants were included in the final analysis (Figure 1). In addition, age and gender were compared between excluded and final analysis subjects, respectively, and there were no significant differences (table not shown). On the basis of data from this study, subjects were classified to three groups (underweight, normal weight, overweight and obesity) along a BMI cut-off (BMI was calculated as weight in kilograms divided by height meters squared). Individuals with a BMI <18.5, 18.5 ~23.9 Kg/m^2 ,≥24 Kg/m^2 were divided into underweight group (n=141), normal weight group (n=1788), and overweight and obesity group (n=1127), respectively. Then, we stratified overweight and obese adults into three groups according to FPG levels: FPG < 5.56 mmol/L, 5.56 ≤ FPG <7.00 mmol/L and FPG ≥ 7.00 mmol/L. Each individual received written information about the aim of the study. If he/she decided to participate, a written informed consent was obtained.

General characteristics

Information on participants' demographic characteristics (age, gender, marital status and education level), health-related characteristics (physical activity, current smoking, current drinking and BMI) and medical history (diabetes, hepatitis B and all other liver diseases) was included in the questionnaire. Marital status was categorized as "Single", "married", and "Divorce or Widowed". Education level was divided into four categories (no school, primary school, middle school and high school or above). Physical activity was categorized as "every day", "more than once a week", "seldom", and "never". Smoking was grouped as "non-smoker", "smoker", and "ex-smoker". Drinking was divided into three categories, "regularly", "seldom", and "never". Smokers were defined as those who smoked one or more cigarettes per day for at least 6 months. Regular drinkers were defined as those who drank alcohol on average more than once a week within the last year. Additionally, because subjects were very few in some dummy variables of marital status, education level, physical activity, current smoking

and current drinking, unmarried and divorced or widowed were considered as single; no school and primary school were merged as primary school or below; Physical activity (yes) included exercise every day and more than once a week; Physical activity (no) included seldom and never exercise; non-smoker and ex-smoker were combined into current smoking (no); current drinking (yes) included those who regularly and seldom drinking.

Anthropometric parameters and laboratory tests

Anthropometric parameters (height and weight) were measured by trained staffs, following a standardized protocol. The data were collected in replicate, and mean values were calculated in the study. After an overnight fasting (at least 8 hours), venous blood samples from participants were obtained and analyzed by PPI automatic biochemical analyzer (Roche Company, Germany) for FPG, albumin (ALB), direct bilirubin (DBIL), indirect bilirubin (IBIL), total bilirubin (TBIL), ALT and AST levels.

Tertiles of erythrocyte parameters levels

Liver enzymes levels were categorized into tertiles [12] on the basis of individual distributions in overweigh and obesity group. ALT: Q1 <17 U/L, Q2=17~25 U/L, Q3 ≥25 U/L; AST: Q1 <20 U/L, Q2=20~24 U/L, Q3 ≥24 U/L.

Procedures

All data were collected on the same day via face-to-face interviews, and blood tests were performed by either a physician or a nurse (the healthcare staff from local Community Health Service Agencies). The interviewers received training to improve their interview skills and standardize the procedures of data collection. Besides, several supervisors were arranged to verify the authenticity of the data.

Patient and public involvement

The role of study subjects in our survey was participants. They were not involved in the development of the research question and outcome measures, the recruitment of subjects and the conduct of the study. After completing this survey, we sent each participant a letter describing detailed results of this study.

Statistics analyses

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago,IL, USA). Data were presented as mean ± SD and frequencies (percentage). A one-way ANOVA was conducted to evaluate differences in age and BMI according to FPG levels, to test mean levels of FPG dependent on the tertiles for ALT and AST in overweight and obesity group. The $\chi 2$ test was used to compare the frequency of general characteristics (categorical variables) according to FPG levels in overweight and obesity group. Partial correlation was performed to investigate the relationship between BMI and related indexes among all participants shown in Table 1, to determine the correlation between FPG levels and liver tests stratified by BMI shown in Table 2.. Multivariate logistic regression analyses were applied to calculate the adjusted ORs for FPG levels associated with liver enzymes levels in overweight and obesity group. (Since there was no association between liver enzymes levels and FPG levels in underweight group and normal group, respectively shown in Table 2, the one-way ANOVA, $\chi 2$ test and multivariate logistic regression were not performed in these two groups.).

Results

Of 3056 subjects, 50.3% (1537/3056) were found to have abnormal FPG, 22.9% (699/3056) have 5.56 ≤ FPG <7.00 mmol/L, and 5.5% (167/3056) have FPG ≥ 7.00 mmol/L. Of 1127 overweight and obese adults, 34.0% (383/1127) were found to have abnormal FPG, 27.5% (310/1156) have 5.56 ≤ FPG <7.00 mmol/L, and 6.5% (73/1127) have FPG ≥ 7.00 mmol/L.

The Partial Correlation and One-Way ANOVA

A total of 3056 adults were included in this study, comprising of 141 (4.6%) individuals with underweight, 1788 (58.5%) individuals with normal weight and 1127 (36.9%) individuals with overweight and obesity. The partial correlation coefficient between BMI and related indexes was shown in Table 1. Every index was significantly correlated with BMI, except for ALB and IBIL. Compared with other indexes, FPG and ALT were more correlated with BMI.

Further, the partial correlation was conducted to explore the relationship between FPG levels and liver tests stratified by BMI (shown in Table 2). Notably, AST and ALT levels were all not associated

with FPG levels in underweight group and normal weight group, respectively, but the significant association was observed in overweight and obesity group. Of the two liver enzymes, ALT (r =0.097, $P < 0.05$) levels had a stronger correlation with FPG levels than AST levels (r =0.070, $P < 0.05$). Mean levels of FPG depending on the baseline tertiles of AST and ALT levels among overweight and obese adults were shown in Figure 2. FPG levels were positively related to ALT levels ($P < 0.05$), but not to AST levels ($P > 0.05$).

Association of general characteristics with FPG

Mean levels of age and BMI, and the frequency of gender, marital status, education level, physical activity, current smoking and current drinking according to FPG levels, in overweight and obesity group were presented in Table 3 Mean age was 60.01 ± 12.67, 63.34 ± 12.06, and 64.75 ± 13.88 in FPG < 5.56 mmol/L, 5.56 ≤ FPG < 7.00 mmol/L and FPG ≥ 7.00 mmol/L groups, respectively. Compared with the FPG < 5.56 mmol/L group, the 5.56 ≤ FPG < 7/00 mmol/L and FPG ≥ 7.00 mmol/L groups displayed significantly higher age ($P < 0.05$). Mean BMI was 26.37 ± 2.18, 26.65 ± 2.21, and 26.92 ± 2.59 in FPG < 5.56 mmol/L, 5.56 ≤ FPG < 7/00 mmol/L and FPG ≥ 7.00 mmol/L groups, respectively. Compared with the FPG < 5.56 mmol/L group, the FPG ≥ 7.00 mmol/L group displayed significantly higher BMI ($P < 0.05$), but this was not true for the 5.56 ≤ FPG < 7.00 mmol/L group ($P > 0.05$).

Multivariate logistic regression analysis model

Adjusted ORs for FPG levels associated with liver enzymes levels in overweight and obesity group were listed in Table 4. Additionally, only those variables that were significantly correlated with FPG levels were presented in Table 4. After adjusting for potential confounders (age, BMI and liver tests), the highest tertiles of ALT levels remained significantly positively correlated with FPG levels with an OR of 2.205 (95% CI: 1.442~3.371) in 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, and with an OR of 2.297 (95% CI: 1.017~5.187) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L, but this was not the same for AST.

Age showed an OR of 1.024 (95% CI: 1.013~1.036) in 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, and 1.033 (95% CI: 1.012–1.054) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L.

However, ALB levels displayed an OR of 0.954 (95% CI: 0.928–0.982) in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L.

Discussion

Partial correlation showed that AST and ALT levels were not associated with FPG levels in underweight group and normal weight group, but the significantly positive association of AST and ALT levels with FPG levels was observed in overweight and obesity group (Table 2). This positive association among overweight and obese adults may be partly explained by the obesity-related diabetes. Fall and his colleagues found a causal effect of adiposity on ALT levels in a Mendelian randomization analysis study. Mechanistically, increased intrahepatic fat content is bi-directionally associated with insulin resistance, which in its turn can lead to increased glucose output from the liver [5, 13-14].

In current study, mean levels of FPG were shown in Figure 2 depending on the baseline tertiles of AST and ALT levels in overweight and obesity group. Our results revealed that the elevated FPG levels were related to the increased levels of liver enzymes, similar to a recent study [15]. Insulin resistance and insulin sensitivity reduction may be the key pathophysiological mechanism of this positive association between liver enzymes levels and FPG levels [8-9]. An epidemiologic study conducted in 10800 middle-aged populations noted that elevated liver enzymes levels were closely related to insulin resistance [16]. Bonnet et al found that liver enzymes activities, even within the normal range, can reduce hepatic insulin extraction among healthy men and women [9].

In the identification of liver injury, ALT is more specific than AST [17]. For instance, Mainous et al, analyzing a nationally representative sample of the noninstitutionalized US population, found that ALT levels, but not AST levels, were independently linked with undiagnosed diabetes (defined as FPG ≥ 126 mg/dl) as well as impaired fasting glucose.(defined as 100 ≤ FPG ≤ 125 mg/dl) [18], consistent with our results in general. In our study, after adjusting for potential confounders (age, BMI and liver tests), ALT levels remained significantly positively correlated with FPG levels both in 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/Land FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L, but this was not the same for AST. It may be that ALT predominantly exists in liver, however, not only is AST found in the liver, but also in cardiac muscle, skeletal, brain and other organs. ALT is the most closely related to liver fat content [19]. Liver fat content, except under certain conditions [20], has been reported to be linked with insulin resistance. Besides, Lu et al clarified that the effect of AST levels on diabetes risk

was partly due to ALT levels [21]. Except for ALT and AST, GGT is also one of liver enzymes. Currently, the association between GGT levels and FPG levels remains controversial. Recent literature reported that a moderate elevation of GGT levels within the normal range was a strong risk predictor for the onset of diabetes [22]， and GGT may be a better predictor of diabetes than ALT [23]. However, Oka et al found that GGT was not associated with the progression to impaired glucose tolerance after adjustment for ALT [24], and a cohort study showed that NAFLD was associated with an increased risk of type 2 diabetes with a higher risk for ALT than GGT [25], Unfortunately, our study did not collect GGT data, and in the future, we will improve this limitation.

In a previous research, ALT levels were associated with FPG levels in FPG ≥ 126 mg/dL vs. FPG < 100 mg /dL (OR: 1.16, 95% CI: 1.00~1.35) [26]. In the present study, the highest tertiles of ALT levels were associated with more than a twofold increase of FPG levels (in 5.56 ≤ FPG < 7.00 mmol/L vs. FPG <5.56 mmol/L) among overweight and obesity populations, independently of conventional risk factors. In depth, the highest tertiles of ALT levels were more significantly correlated with FPG levels in FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L (Table 4), similar to an early study [27]. Gonzálezpérez et al reported that compared to normal ALT levels, the relative risk (RR) for the incidence of impaired fasting glucose (100 ≤ FPG ≤ 125 mg/dl) and diabetes (FPG ≥ 126 mg/dl) depending on the levels of ALT was 3.09 in borderline elevated ALT levels and 1.59 in elevated ALT levels [27]. NAFLD may play an important role in the relationship between ALT levels and FPG levels among overweight and obesity populations. It has been found that patients with NAFLD are at increased risk for developing type 2 diabetes. Liver fat content was inversely associated with hepatic, adipose tissue and muscle insulin sensitivity and this might contribute to the increased risk of type 2 diabetes [28]. Additonally, NAFLD can result in an elevated ALT levels [25]. The following mechanisms may be also regarded as the causes of the association between elevated ALT levels and the increased risk of elevated FPG levels. 1) Elevated ALT levels reflected potential chronic inflammation and increased oxidative stress, while chronic inflammation and oxidative stress appeared to be involved in the pathogenesis of NAFLD [28], which may impair insulin signaling in the liver and other organ tissues [9, 29]; 2) Elevated ALT levels could reflect life-long hepatitis virus infection, which can result in diabetes [30]; 3) The testosterone levels may be the mediator between ALT levels and the risk of diabetes. Researchers have revealed the role of low testosterone in diabetes [31], and that poor liver function may reduce testosterone production [32].s

Our study was conducted in the Community Health Service Agencies, in Guangdong Province of China, and it may imply that the generalisability of our results is limited to this region. Additionally, participants with a history of diabetes, hepatitis B, all other liver diseases were excluded from the study, so our results are not applicable to these subjects.

Except GGT was not included in this study, limitation of the current study included the absence of imaging studies. Recent studies noted that imaging studies will likely provide a new opportunity for investigating the association of the liver with diabetic disease [33-34]. Then, supplementary information about the blood lipid, diseases types and medication history of subjects was not collected. Hence, some factors such as cholesterol, triglycerides levels, could not be included in the adjustments of our multivariate logistic regression analyses. In addition, our study design was cross-sectional, and direct causation cannot be concluded from the results.

Conclusions

The association of liver enzymes levels with FPG levels differed along a BMI cut-off. ALT levels were significantly positively associated with FPG levels in overweight and obesity group, but not in underweight group and normal weight group; AST levels were not associated with FPG levels in all groups. These findings have important clinical implications for health makers. Liver enzymes may serve as effective indices for the early detection of diabetes high-risk individuals on a BMI dependent basis.

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Contributors: LLH, YPJ and PXW conducted the data analyses. LLH and DHG drafted the manuscript. DHG, HYX, STT and XXW finalized the manuscript with inputs from all authors. All authors contributed to the development of the study framework, interpretation of the results, revisions of successive drafts of the manuscript, and approved the version submitted for publication.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

Ethical approval: The protocol of this study was approved by the ethics committee of the Community Health Service Agencies of Liaobu town, Dongwan city, Guangdong province. The ethical code is 20130410.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Data sharing statement This database is first used in this study. The database belongs to our team, and if shared, you need to get their permission.

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Figure Legends

Fig 1 Flow chart in the selection of study population

Fig 2 Mean levels of fasting plasma glucose (FPG) levels depending on the baseline tertiles of aspartate aminotransferase (AST) and alanine transaminase (ALT) levels in overweight and obesity group

Table 1 Partial correlation between BMI and related indexes (n=3056)

Related indexes	Partial correlation coefficient (Controlling age, gender)	*p*
FPG	0.077	<0.001***
ALB	-0.010	0.573
DBIL	-0.049	0.008**
IBIL	-0.004	0.833
TBIL	-0.038	0.035*
ALT	0.165	<0.001***
AST	0.037	0.040*

BMI: body mass index; FPG: fasting plasma glucose; ALB: albumin; DBIL: direct bilirubin; IBIL: indirect bilirubin; TBIL: total bilirubin; ALT: alanine transaminase; AST: aspartate aminotransferase; Except for gender, all the variables (age BMI, FPG, ALB, DBIL, IBIL, TBIL, ALT and AST) in the partial correlation coefficient were continuous variables; * p <0.05, **p <0.01,*** p <0.001.

Table 2 Partial correlation between FPG levels and liver tests stratified by BMI (n=3056)

Liver tests	Underweight group (correlation coefficient, n=141)	Normal weight group (correlation coefficient, n=1788)	Overweight and obesity group (correlation coefficient, n=1127)
ALB	-0.042	-0.057*	-0.097**
DBIL	0.021	-0.024	0.033
IBIL	-0.005	-0.010	-0.111***
TBIL	-0.025	-0.035	-0.068*
ALT	0.011	0.013	0.078**
AST	-0.034	-0.039	0.070*

Partial correlation coefficient: controlling age, gender and BMI; BMI: body mass index; FPG: fasting plasma glucose; ALB: albumin; DBIL: direct bilirubin; IBIL: indirect bilirubin； TBIL: total bilirubin; ALT: alanine transaminase; AST: aspartate aminotransferase; Except for gender, all the variables (age BMI, FPG, ALB, DBIL, IBIL, TBIL, ALT and AST) in the partial correlation coefficient were continuous variables; * p <0.05, **p <0.01,*** p <0.001.

Table 3 General characteristics associated with FPG levels in overweight and obesity group (n=1127)

Variables	FPG < 5.56 (n=744)	5.56 ≤ FPG < 7.00 (n=310)	FPG ≥ 7.00 (n=73)
Age, years (m, SD)	60.01 ± 12.67	63.34 ± 12.06*	64.75 ± 13.88**
Gender (n, %)			
Male	255 (34.4)	106 (34.2)	35 (47.9)
Female	489 (65.7)	204 (65.8)	38 (52.1)
Marital status (n, %)			
Single	82 (11.4)	23 (7.7)	10 (14.7)
Married	635 (88.6)	275 (92.3)	58 (85.3)

Education level (n, %)			
Primary school or below	161 (37.9)	86 (43,0)	18 (38.3)
Middle school	221 (52.0)	91 (45.5)	25 (53.2)
High school or above	43 (10.1)	23 (11.5)	4 (8.5)
Physical activity (n, %)			
Yes	202 (27.2)	101 (32.6)	21 (28.8)
No	542 (72.8)	209 (67.4)	52 (71.2)
Current smoking (n, %)			
Yes	33 (4.4)	15 (4.8)	8 (11.0)
No	711 (95.6)	295 (95.2)	65 (89.0)
Current drinking (n, %)			
Yes	13 (1.7)	9 (2.9)	3 (4.1)
No	731 (98.3)	301 (97.1)	70 (95.9)
BMI, Kg/m^2 (m, SD)	26.37 ± 2.18	26.65 ± 2.21	26.92 ± 2.59**

Data were presented as mean (SD) or n (%); FPG: fasting plasma glucose; BMI: body mass index; Single included Single: unmarried, divorced or widowed; Primary school or below: no school, primary school; Physical activity (yes): every day, More than once a week; Physical activity (no): seldom, never; Current smoking (no): non-smoker, ex-smoker; Current drinking (yes): regularly, seldom; *P < 0.05 5.56 ≤ FPG < 7.00 mmol/L vs FPG < 5.56 mmol/L; **P < 0.05 FPG ≥ 7.00 mmol/L vs FPG < 5.56 mmol/L

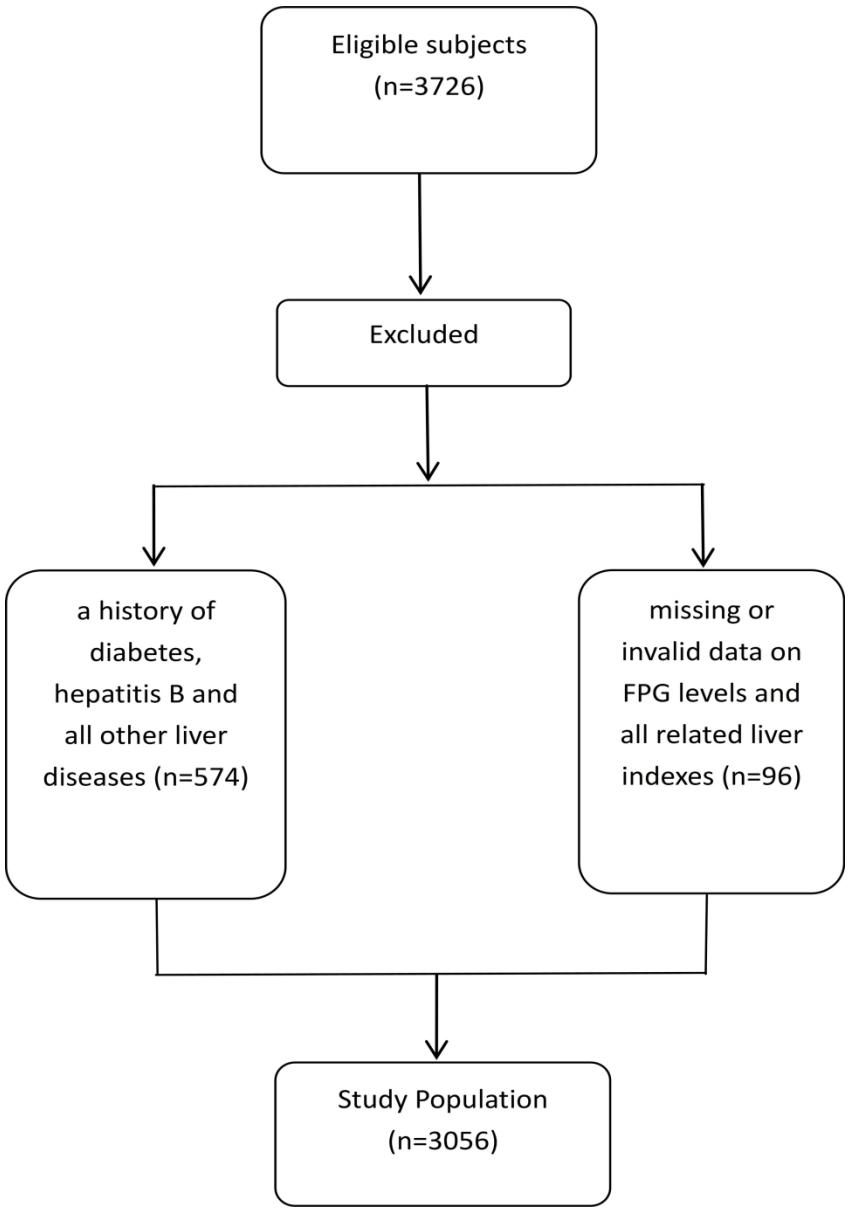
Table 4. Odds ratios for FPG elevation by liver enzymes levels in overweight and obesity group (n=1127)

Variables	5.56 ≤ FPG < 7.00 VS FPG< 5.56		FPG≥ 7.00 VS FPG<5.56	
	OR (95% CI)	P	OR (95% CI)	P
Age	1.024 (1.013~1.036)	<0.001***	1.033 (1.012~1.054)	0.002**
ALB	—		0.954 (0.928~0.982)	0.001**
ALT				
Q1	Reference			
Q2	1.357 (0.936~1.967)	0.108	1.677 (0.799~3516)	0.171

	Q3	2.205 (1.442~3.371)	<0.001***	2.297 (1.017~5.187)	0.045*

Statistical analysis by multivariate logistic regression (adjusted for age, BMI (body mass index) and liver tests); OR: odds ratio; CI: confidence interval; FPG: fasting plasma glucose; ALB: albumin; IBIL: indirect bilirubin; ALT: alanine transaminase.

Goodness-of-fit results: Pearson $\chi 2$ test, P=0.465; Deviance$\chi 2$ test, P=1.000.



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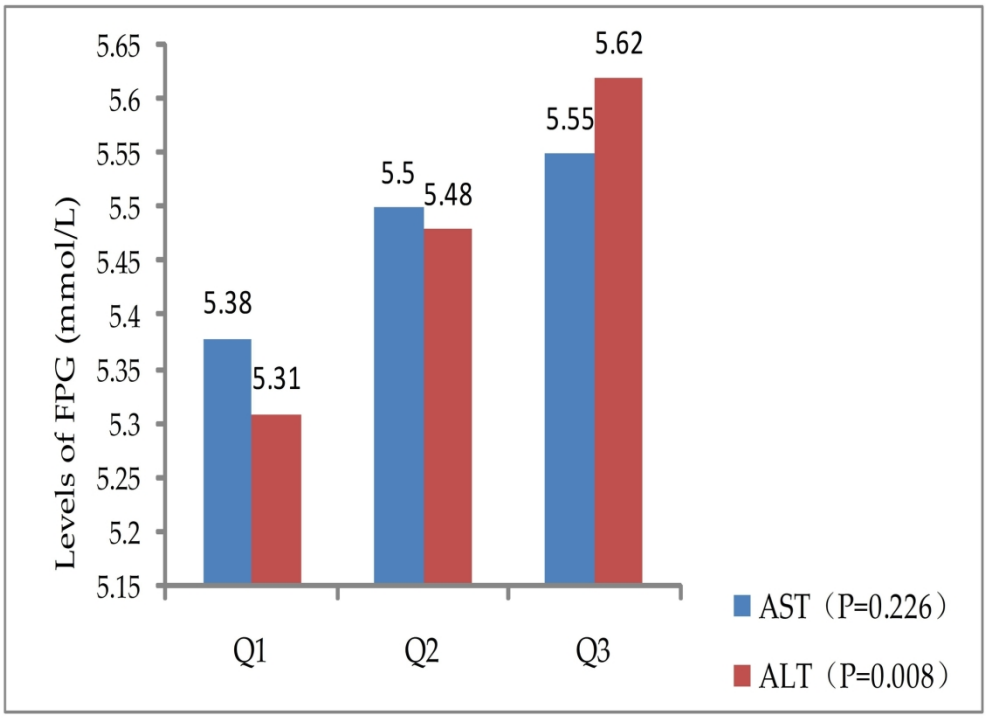

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 4)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 5)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants (Page 5)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 5-6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 5-6)
Bias	9	Describe any efforts to address potential sources of bias (Page 6, 8)
Study size	10	Explain how the study size was arrived at (Page 5)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page 5, 6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 7)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 7)
		(*c*) Explain how missing data were addressed (Page 5)
		(*d*) If applicable, describe analytical methods taking account of sampling strategy (not applicable)
		(*e*) Describe any sensitivity analyses (Page 7)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Page 7, Table 1-3)
		(b) Give reasons for non-participation at each stage (not applicable)
		(c) Consider use of a flow diagram (Figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 7-8, Table 1-3)
		(b) Indicate number of participants with missing data for each variable of interest (Not applicable)
Outcome data	15*	Report numbers of outcome events or summary measures (Page 7-8, Table 3-4)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Page 7-8, Table 4)

		(*b*) Report category boundaries when continuous variables were categorized (Page 5-6)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (Not applicable)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Not applicable)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 8-10)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 9-11)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 9-10)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 10)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 11)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association of liver enzymes levels with fasting plasma glucose levels in Southern China: a cross-sectional study

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Association of liver enzymes levels with fasting plasma glucose levels in Southern China: a cross-sectional study

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Abstract

Objective: According to several studies, liver enzymes levels are associated with fasting plasma glucose (FPG) levels. However, the association stratified by body mass index (BMI) remains to be elucidated, especially in Southern China. Therefore, the aim of this study was to investigate the correlation between liver enzymes levels and FPG levels stratified by BMI in Southern China.

Design: Cross-sectional study

Participants and setting: 3056 individuals participated in real-time interviews and blood tests in Southern China. Participants were divided into three groups (underweight, normal weight, and overweight or obesity) based on BMI cut-offs.

Main outcome measured: Partial correlation analysis was performed to investigate the relationship between FPG levels and liver tests. Multivariate logistic regression analyses were applied to calculate the adjusted ORs for FPG levels based on liver enzymes levels.

Results: There was no association between liver enzymes and FPG either in the underweight group or in the normal weight group, however, a significant correlation was observed in the overweight or obesity group (alanine transaminase (ALT), $P < 0.01$; aspartate aminotransferase (AST), $P < 0.05$). After adjusting for confounding factors, the highest tertiles of ALT still remained significantly positively related to FPG levels in the overweight or obesity group, with an OR of 2.205 (95% CI: 1.442~3.371) for the $5.56 \leq$ FPG < 7.00 mmol/L vs. the FPG < 5.56 mmol/L group, and with an OR of 2.297 (95% CI: 1.017~5.187) for the FPG ≥ 7.00 mmol/L vs. the FPG < 5.56 mmol/L group, but this correlation was not found for AST.

Conclusions: The association of liver enzymes levels with FPG levels differed based on different BMI cut-offs. ALT levels were significantly positively associated with FPG levels in the overweight or obesity group, but not in the other two groups; AST levels were not associated with FPG levels in any group.

Keywords: Liver enzymes; Fasting plasma glucose; Southern China; Cross-sectional study

Strengths and limitations of this study

- A large sample of subjects was enrolled in this survey.
- To the best of our knowledge, this is the first study to explore the correlation between liver enzymes levels and FPG levels stratified by BMI among people who had not been diagnosed with diabetes before this survey in Southern China.
- The present study was designed as a cross-sectional study; therefore, direct causation cannot be concluded from the results.
- Supplementary information about γ-glutamyltransferase (GGT) levels, imaging studies, cholesterol, and triglycerides was not collected; therefore, it could not be determined whether these factors were associated with FPG. Addtionally, some factors such as cholesterol and triglycerides levels could not be adjusted in the a multivariate logistic regression analyses.

Introduction

Diabetes, a prevalent chronic disease, has emerged as a major public health concern due its increased prevalence in many countries, affecting approximately 382 million people worldwide [1]. China, the largest developing country, also has a high incidence of diabetes. Recently, a survey revealed that more than one in ten Chinese adults was affected by diabetes [2]. Therefore, the early identification of individuals at high risk of diabetes is essential for decreasing the prevalence of diabetes.

The liver is the site of glycogen synthesis and gluconeogenesis, which together with the pancreas, muscle, adipose tissue and other organs, plays an important role in maintaining the stable level of blood glucose[3-4]. Liver enzymes, the most common markers of liver function in the clinic, include alanine transaminase (ALT) and aspartate aminotransferase. (AST) [5-7]. Related studies suggested that the elevation of liver enzymes levels was indicative of insulin sensitivity reduction, insulin resistance, and type 2 diabetes development [8-10]. Fasting plasma glucose (FPG) is the most commonly used index to monitor the occurrence of early type 2 diabetes, which is of great significance in the prevention of diabetes. Although previous studies have reported that liver enzymes levels were significantly associated with FPG levels [3,10-11], the evidence remained insufficient, because the reported results were inconsistent in terms of the population studied, such as populations from different regions, and populations with different body mass indexes (BMIs). In addition, as we know, almost all related studies regarded BMI as only a covariate in the investigation of the relationship between liver enzymes levels and FPG levels in the general population, and few studies were conducted in Southern China. Therefore, the aim of this study was to determine the association of liver enzymes levels with FPG levels, stratified by BMI, among people who had not been diagnosed with diabetes before this survey, in Southern China. If elevated liver enzymes levels are significantly associated with an increase in FPG levels, there might be implications in terms of considering liver enzymes as effective molecular markers for the early detection of individuals at high risk of diabetes with different BMI cut-off points, and health policy makers can develop targeted interventions to prevent the early occurrence of type 2 diabetes according to different BMI cut-off points.

Materials and Methods

Study population

This cross-sectional study was conducted in the Guangdong Province of China in 2014. Initially, 3726 healthy inhabitants who underwent a general health examination (mean age: 60.32 years, ≥18 years old) were recruited from local Community Health Service Agencies. All participants completed the survey, and the overall response rate was 100%.The health examination included recording of general characteristics, medical history, anthropometric parameters and laboratory tests. After excluding subjects (n=574) with a history of diabetes, hepatitis B and all other liver diseases, and subjects (n=96) with missing or invalid data on FPG levels or liver related indexes, 3056 eligible participants were included in the final analysis (Figure 1). In addition, age and sex were compared between the excluded participants and those included in the final analysis, respectively, and there were no significant differences (table was presented in supplementary file). Based on the basis of data from this study, subjects were classified into three groups (underweight, normal weight, and overweight or obesity) based on BMI cut-offs (BMI was calculated as weight in kilograms divided by height meters squared). Individuals with a BMI <18.5 kg/m^2, 18.5 ~23.9 kg/m^2 or ≥24 kg/m^2 were categorized into the underweight group (n=141), the normal weight group (n=1788), and the overweight or obesity group (n=1127), respectively. Then, we stratified overweight and obese adults into three groups according to FPG levels: FPG < 5.56 mmol/L, 5.56 ≤ FPG <7.00 mmol/L and FPG ≥ 7.00 mmol/L. Each individual received written information about the aim of the study. If he/she decided to participate, a written informed consent was obtained.

General characteristics

Information on participants' demographic characteristics (age, sex, marital status and education level), health-related characteristics (physical activity, current smoking, alcohol consumption and BMI) and medical history (diabetes, hepatitis B and all other liver diseases) was included in the questionnaire. Marital status was categorized as "single", "married", and "divorce or widowed". Education level was divided into four categories (no school, primary school, middle school and high school or above). Physical activity was categorized as "every day", "more than once a week", "seldom", and "never". Smoking status was categorized as "non-smoker", "smoker", and "ex-smoker". Alcohol consumption was divided into three categories, "regularly", "seldom", and "never". Smokers were defined as those who smoked one or more cigarettes per day for at least 6 months. Regular alcohol consumers were defined as those who consumed alcohol on average more than once a week within the last year.

Additionally, because very few subjects were included in some dummy variables categories of marital status, education level, physical activity, current smoking and alcohol consumption, unmarried and divorced or widowed were considered single; no school and primary school were merged as primary school or below; physical activity (yes) included those who exercised every day or more than once a week; physical activity (no) included those who seldomly or never exercised; non-smokers and ex-smokers were combined into current smoking (no); and alcohol consumption (yes) included those who regularly and seldomly consumed alcohol.

Anthropometric parameters and laboratory tests

Anthropometric parameters (height and weight) were measured by trained staffs, following a standardized protocol. The data were collected in replicate, and mean values were calculated in the study. After overnight fasting (at least 8 hours), venous blood samples from participants were obtained and analyzed by a PPI automatic biochemical analyzer (Roche Company, Germany) for FPG, albumin (ALB), direct bilirubin (DBIL), indirect bilirubin (IBIL), total bilirubin (TBIL), ALT and AST levels.

Tertiles of liver enzymes levels

Liver enzymes levels were categorized into tertiles [12] based on individual distributions in the overweight or obesity group. ALT: Q1 <17 U/L, Q2=17~25 U/L, Q3 ≥25 U/L; AST: Q1 <20 U/L, Q2=20~24 U/L, Q3 ≥24 U/L.

Procedures

All data were collected on the same day via face-to-face interviews, and blood tests were performed by either a physician or a nurse (the healthcare staff from the local Community Health Service Agencies). The interviewers received training to improve their interview skills and standardize the procedures of data collection. In addition, several supervisors were selected to verify the authenticity of the data.

Patient and public involvement

The role of study subjects in our survey was participants. They were not involved in the development of the research question and outcome measures, the recruitment of subjects and the conduct of the

study. After completing this survey, we sent each participant a letter describing detailed results of this study.

Statistics analyses

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago,IL, USA). Data are presented as the mean ± SD and frequencies (percentage). A one-way ANOVA was conducted to evaluate differences in age and BMI according to FPG levels, and to test mean levels of FPG based on the tertiles of ALT and AST in the overweight or obesity group. The $\chi 2$ test was used to compare the frequency of general characteristics (categorical variables) according to FPG levels in the overweight or obesity group. Partial correlation analysis was performed to investigate the relationship between BMI and related indexes among all participants, as shown in Table 1, to determine the correlation between FPG levels and liver tests stratified by BMI, as shown in Table 2.. Multivariate logistic regression analyses were applied to calculate the adjusted ORs for FPG levels associated with liver enzymes levels in the overweight or obesity group. (since there was no association between liver enzymes levels and FPG levels in underweight group and normal group, respectively, as shown in Table 2, one-way ANOVA, $\chi 2$ test and multivariate logistic regression were not performed for these two groups.).

Results

Of 3056 subjects, 50.3% (1537/3056) were found to have abnormal FPG levels, 22.9% (699/3056) had 5.56 ≤ FPG <7.00 mmol/L, and 5.5% (167/3056) had FPG ≥ 7.00 mmol/L. Of 1127 overweight and obese adults, 34.0% (383/1127) were found to have abnormal FPG levels, 27.5% (310/1156) had 5.56 ≤ FPG <7.00 mmol/L, and 6.5% (73/1127) had FPG ≥ 7.00 mmol/L.

The Partial Correlation and One-Way ANOVA

A total of 3056 adults were included in this study, comprising 141 (4.6%) individuals who were underweight, 1788 (58.5%) individuals with normal weight and 1127 (36.9%) individuals with overweight or obesity. The partial correlation coefficients between BMI and related indexes were shown in Table 1. Every index was significantly correlated with BMI, except for ALB and IBIL. Compared with other indexes, FPG and ALT were more strongly correlated with BMI.

Further, a partial correlation analysis was conducted to explore the relationship between FPG levels and liver tests stratified by BMI (shown in Table 2). Notably, AST and ALT levels were not associated with FPG levels in the underweight group and normal weight group, respectively, but a significant association was observed in the overweight or obesity group. Of the two liver enzymes, ALT (r =0.097, $P < 0.05$) was more strongly correlated with FPG levels than AST (r =0.070, $P < 0.05$). The mean FPG levels by baseline tertiles of AST and ALT levels among overweight and obese adults were shown in Figure 2. The FPG levels were positively related to ALT levels ($P < 0.05$), but not to AST levels ($P > 0.05$).

Association of general characteristics with FPG

Mean age and BMI, and the frequency of sex, marital status, education level, physical activity, current smoking and alcohol consumption according to FPG levels in the overweight or obesity group are presented in Table 3 The mean ages were 60.01 ± 12.67, 63.34 ± 12.06, and 64.75 ± 13.88 in the FPG < 5.56 mmol/L, 5.56 ≤ FPG < 7.00 mmol/L and FPG ≥ 7.00 mmol/L groups, respectively. Compared with the FPG < 5.56 mmol/L group, the 5.56 ≤ FPG < 7/00 mmol/L and FPG ≥ 7.00 mmol/L groups displayed significantly higher ages ($P < 0.05$). The mean BMIs were 26.37 ± 2.18, 26.65 ± 2.21, and 26.92 ± 2.59 in the FPG < 5.56 mmol/L, 5.56 ≤ FPG < 7/00 mmol/L and FPG ≥ 7.00 mmol/L groups, respectively. Compared with the FPG < 5.56 mmol/L group, the FPG ≥ 7.00 mmol/L group displayed a significantly higher BMI ($P < 0.05$), but this was not true for the 5.56 ≤ FPG < 7.00 mmol/L group ($P > 0.05$).

Multivariate logistic regression analysis model

The adjusted ORs for FPG levels associated with liver enzymes levels in the overweight or obesity group are listed in Table 4. Additionally, only those variables that were significantly correlated with FPG levels are presented in Table 4. After adjusting for potential confounders (age, BMI and liver tests), the highest tertiles of ALT levels remained significantly positively correlated with FPG levels with an OR of 2.205 (95% CI: 1.442~3.371) for 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, and with an OR of 2.297 (95% CI: 1.017~5.187) for FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L, but this correlation was not found for AST.

Age had an OR of 1.024 (95% CI: 1.013~1.036) for 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/L, and of 1.033 (95% CI: 1.012–1.054) for FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L. However, ALB levels displayed an OR of 0.954 (95% CI: 0.928–0.982) for FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L.

Discussion

Partial correlation analysis showed that AST and ALT levels were not associated with FPG levels in the underweight group and the normal weight group, but a significantly positive association of AST and ALT levels with FPG levels was observed in the overweight or obesity group (Table 2). This positive association in the group of overweight or obese adults may be partly explained by the obesity-related diabetes. Fall and his colleagues found a causal effect of adiposity on ALT levels in a Mendelian randomization analysis study[13]. Mechanistically, increased intrahepatic fat content is bi-directionally associated with insulin resistance, which in turn can lead to increased glucose output from the liver [5, 14-15].

In current study, mean levels of FPG are shown in Figure 2 based on the baseline tertiles of AST and ALT levels in the overweight or obesity group. Our results revealed that the elevated FPG levels were related to the increased levels of liver enzymes, which is similar to the result of a recent study [16]. Insulin resistance and reduced insulin sensitivity may be the key pathophysiological mechanism underlying this positive association between liver enzymes levels and FPG levels [8-9]. An epidemiologic study conducted with 10800 middle-aged participants noted that elevated liver enzymes levels were closely related to insulin resistance [17]. Bonnet et al found that liver enzymes activities, even within the normal range, can reduce hepatic insulin extraction among healthy men and women [9].

In regard to the identification of liver injury, ALT is more specific than AST [18]. For instance, Mainous et al, in the analysis of a nationally representative sample of the noninstitutionalized US population, found that ALT levels, but not AST levels, were independently linked with undiagnosed diabetes (defined as FPG ≥ 126 mg/dl) as well as impaired fasting glucose.(defined as 100 ≤ FPG ≤ 125 mg/dl) [19], which is consistent with our results in general. In our study, after adjusting for potential confounders (age, BMI and liver tests), ALT levels remained significantly positively correlated with FPG levels both for 5.56 ≤ FPG < 7.00 mmol/L vs. FPG < 5.56 mmol/Land FPG ≥ 7.00

mmol/L vs. FPG < 5.56 mmol/L, but this correlation was not found for AST. An explanation may be that ALT predominantly exists in liver, whereas AST is found in the liver and also in cardiac and skeletal muscle, the brain and other organs. ALT is the most closely related to liver fat content [20]. Liver fat content, except under certain conditions [21], has been reported to be linked with insulin resistance. In addition, Lu et al clarified that the effect of AST levels on diabetes risk was partly due to ALT levels [22]. In addition to ALT and AST, GGT is also a liver enzyme. Currently, the association between GGT levels and FPG levels remains controversial. Recent literature has reported that a moderate elevation in GGT levels within the normal range was a strong risk predictor for the onset of diabetes [23], and GGT may be a better predictor of diabetes than ALT [24]. However, Oka et al found that GGT was not associated with the progression to impaired glucose tolerance after adjustment for ALT [25], and a cohort study showed that NAFLD was associated with an increased risk of type 2 diabetes with a higher risk associated with ALT than with GGT [26]. Unfortunately, our study did not collect GGT data, and in the future, we will address this limitation.

In a previous study, ALT levels were associated with FPG levels for FPG ≥ 126 mg/dL vs. FPG < 100 mg /dL (OR: 1.16, 95% CI: 1.00~1.35) [27]. In the present study, the highest tertiles of ALT levels were associated with a more than twofold increase in FPG levels (for 5.56 ≤ FPG < 7.00 mmol/L vs. FPG <5.56 mmol/L) among overweight or obese individuals, independent of conventional risk factors. In depth, the highest tertiles of ALT levels were more significantly correlated with FPG levels for FPG ≥ 7.00 mmol/L vs. FPG < 5.56 mmol/L (Table 4), which was similar to the result of an early study [28]. Gonzálezpérez et al reported that compared to normal ALT levels, the relative risk (RR) for the incidence of impaired fasting glucose (100 ≤ FPG ≤ 125 mg/dl) and diabetes (FPG ≥ 126 mg/dl) based on the level of ALT was 3.09 for borderline elevated ALT levels and 1.59 for elevated ALT levels [28]. NAFLD may play an important role in the relationship between ALT levels and FPG levels among overweight or obese individuals. It has been found that patients with NAFLD are at increased risk for developing type 2 diabetes. Liver fat content was inversely associated with hepatic, adipose tissue and muscle insulin sensitivity, which might contribute to the increased risk of type 2 diabetes [29]. Additionally, NAFLD can result in an elevated ALT levels [26]. The following mechanisms may also be regarded as the underlying causes of the association between elevated ALT levels and the increased risk of elevated FPG levels. 1) Elevated ALT levels reflected potential chronic inflammation and increased oxidative stress, and chronic inflammation and oxidative stress appeared to

be involved in the pathogenesis of NAFLD [29], which may impair insulin signaling in the liver and other organ tissues [9, 30]; 2) Elevated ALT levels could reflect life-long hepatitis virus infection, which can result in diabetes [31]; 3) Testosterone levels may be the mediator between ALT levels and the risk of diabetes. Researchers have revealed the role of low testosterone in diabetes [32], and that poor liver function may reduce testosterone production [33].s

Our study was conducted in Community Health Service Agencies, in Guangdong Province of China, which may imply that the generalizability of our results is limited to this region. Additionally, participants with a history of diabetes, hepatitis B, or other liver diseases were excluded from the study, so our results are not applicable to these subjects.

In addition to GGT not being included in this study, the limitations of the current study included the absence of imaging studies. Recent studies have noted that imaging studies will likely provide a new opportunity for investigating the association of the liver function with diabetic disease [34-35]. Then, supplementary information about the blood lipids, disease types and medication history of subjects was not collected. Hence, some factors such as cholesterol and triglyceride levels, could not be included as covariates in our multivariate logistic regression analyses. In addition, our study design was cross-sectional, and direct causation cannot be concluded from the results.

Conclusions

The association of liver enzymes levels with FPG levels differed based on a BMI cut-off. ALT levels were significantly positively associated with FPG levels in the overweight or obesity group, but not in the underweight group and normal weight groups; AST levels were not associated with FPG levels in any group. These findings have important clinical implications for health policy makers. Liver enzymes may serve as effective indexes for the early detection of individuals at high risk of diabetes on a BMI-dependent basis.

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Contributors: LLH, YPJ and PXW conducted the data analyses. LLH and DHG drafted the manuscript. DHG, HYX, STT and XXW finalized the manuscript with inputs from all authors. All authors contributed to the development of the study framework, interpretation of the results, and revisions of successive drafts of the manuscript, and approved the version submitted for publication.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

Ethical approval: The protocol of this study was approved by the ethics committee of the Community Health Service Agencies of Liaobu town, Dongwan city, Guangdong province. The ethical code is 20130410.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Data sharing statement This database was first used in this study. The database belongs to our team, and permission is required for the database to be shared..

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Figure Legends

Fig 1 Flow chart in the selection of study population

Fig 2 Mean levels of fasting plasma glucose (FPG) levels based on tertiles of aspartate aminotransferase (AST) and alanine transaminase (ALT) levels in the overweight or obesity group

Table 1 Partial correlation analysis between BMI and related indexes (n=3056)

Related indexes	Partial correlation coefficient (Controlling age, gender)	*p*
FPG	0.077	<0.001***
ALB	-0.010	0.573
DBIL	-0.049	0.008**
IBIL	-0.004	0.833
TBIL	-0.038	0.035*
ALT	0.165	<0.001***
AST	0.037	0.040*

BMI: body mass index; FPG: fasting plasma glucose; ALB: albumin; DBIL: direct bilirubin; IBIL: indirect bilirubin; TBIL: total bilirubin; ALT: alanine transaminase; AST: aspartate aminotransferase; Except for gender, all the variables (age BMI, FPG, ALB, DBIL, IBIL, TBIL, ALT and AST) in the partial correlation coefficient were continuous variables; * p <0.05, ** p <0.01,*** p <0.001.

Table 2 Partial correlation analysis between FPG levels and liver tests stratified by BMI (n=3056)

Liver tests	Underweight group (correlation coefficient, n=141)	Normal weight group (correlation coefficient, n=1788)	Overweight or obesity group (correlation coefficient, n=1127)
ALB	-0.042	-0.057*	-0.097**

DBIL	0.021	-0.024	0.033
IBIL	-0.005	-0.010	-0.111***
TBIL	-0.025	-0.035	-0.068*
ALT	0.011	0.013	0.078**
AST	-0.034	-0.039	0.070*

Partial correlation coefficient: controlling age, gender and BMI; BMI: body mass index; FPG: fasting plasma glucose; ALB: albumin; DBIL: direct bilirubin; IBIL: indirect bilirubin； TBIL: total bilirubin; ALT: alanine transaminase; AST: aspartate aminotransferase; Except for sex, all the variables (age BMI, FPG, ALB, DBIL, IBIL, TBIL, ALT and AST) in the partial correlation anaysis were continuous variables; * p <0.05, ** p <0.01,*** p <0.001.

Table 3 General characteristics associated with FPG levels in the overweight or obesity group (n=1127)

Variables	FPG < 5.56 (n=744)	5.56 ≤ FPG < 7.00 (n=310)	FPG ≥ 7.00 (n=73)
Age, years (m, SD)	60.01 ± 12.67	63.34 ± 12.06*	64.75 ± 13.88**
sex (n, %)			
Male	255 (34.4)	106 (34.2)	35 (47.9)
Female	489 (65.7)	204 (65.8)	38 (52.1)
Marital status (n, %)			
Single	82 (11.4)	23 (7.7)	10 (14.7)
Married	635 (88.6)	275 (92.3)	58 (85.3)
Education level (n, %)			
Primary school or below	161 (37.9)	86 (43,0)	18 (38.3)
Middle school	221 (52.0)	91 (45.5)	25 (53.2)
High school or above	43 (10.1)	23 (11.5)	4 (8.5)
Physical activity (n, %)			

Yes	202 (27.2)	101 (32.6)	21 (28.8)
No	542 (72.8)	209 (67.4)	52 (71.2)
Current smoking (n, %)			
Yes	33 (4.4)	15 (4.8)	8 (11.0)
No	711 (95.6)	295 (95.2)	65 (89.0)
Alcohol consumption (n, %)			
Yes	13 (1.7)	9 (2.9)	3 (4.1)
No	731 (98.3)	301 (97.1)	70 (95.9)
BMI, Kg/m^2 (m, SD)	26.37 ± 2.18	26.65 ± 2.21	26.92 ± 2.59**

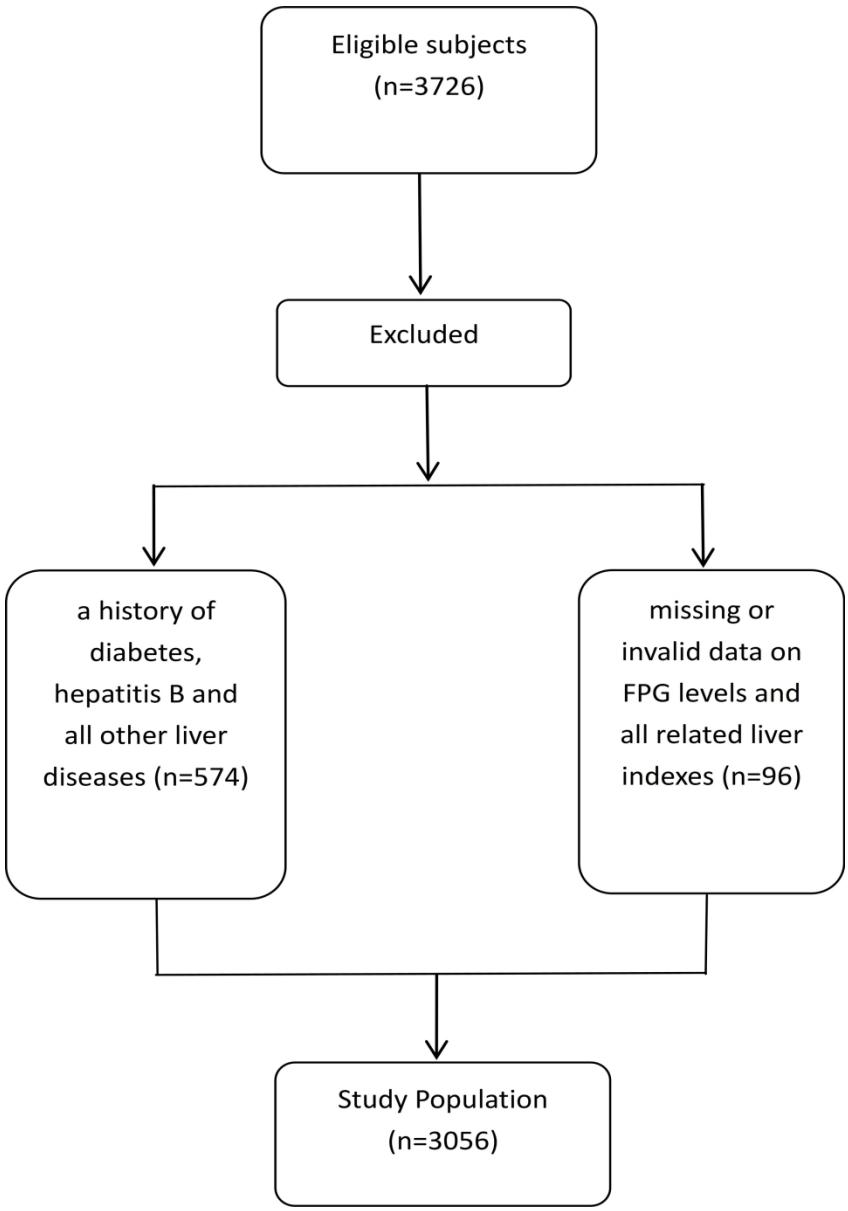
Data are presented as the mean (SD) or n (%); FPG: fasting plasma glucose; BMI: body mass index. Single: unmarried, divorced or widowed; primary school or below: no school, primary school; physical activity (yes): every day, more than once a week; physical activity (no): seldom, never; current smoking (no): non-smoker, ex-smoker; alcohol consumption (yes): regularly, seldom; *P < 0.05 5.56 ≤ FPG < 7.00 mmol/L vs FPG < 5.56 mmol/L; **P < 0.05 FPG ≥ 7.00 mmol/L vs FPG < 5.56 mmol/L

Table 4. Odds ratios for FPG elevation by liver enzymes levels in the overweight or obesity group (n=1127)

Variables	5.56 ≤ FPG < 7.00 VS FPG< 5.56		FPG≥ 7.00 VS FPG<5.56	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.024 (1.013~1.036)	<0.001***	1.033 (1.012~1.054)	0.002**
ALB	—		0.954 (0.928~0.982)	0.001**
ALT				
Q1	Reference			
Q2	1.357 (0.936~1.967)	0.108	1.677 (0.799~3516)	0.171
Q3	2.205 (1.442~3.371)	<0.001***	2.297 (1.017~5.187)	0.045*

Statistical analysis by multivariate logistic regression (adjusted for age, BMI (body mass index) and liver tests); OR: odds ratio; CI: confidence interval; FPG: fasting plasma glucose; ALB: albumin; IBIL: indirect bilirubin; ALT: alanine transaminase.

Goodness-of-fit results: Pearson χ2 test, P=0.465; Devianceχ2 test, P=1.000.



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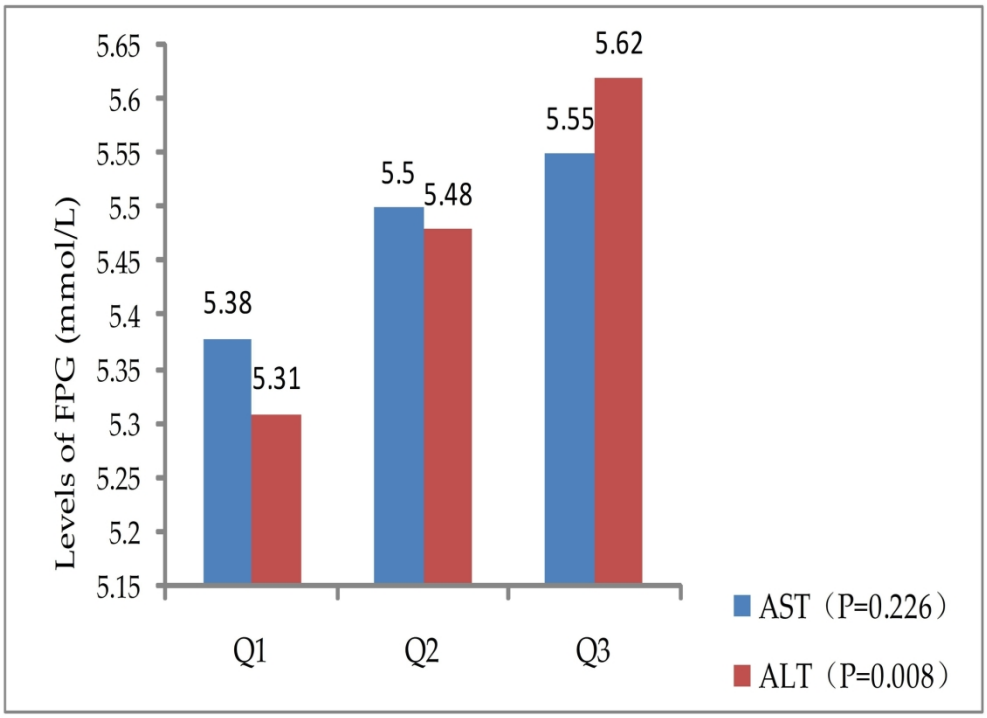

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Comparison of age and gender between excluded and final analysis subjects.

Variables	Excluded subjects (n=670)	Final analysis subjects (n=3056)	*P* value
Age, years (m, SD)	60.45 ± 14.13	60.44 ± 14.11	0.988
Gender (n, %)			0.103
Male	254 (37.9)	1057 (34.6)	
Female	416 (62.1)	1999 (65.4)	

Data were presented as mean (SD) or n (%);

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 4)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 5)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants (Page 5)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 5-6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 5-6)
Bias	9	Describe any efforts to address potential sources of bias (Page 6, 8)
Study size	10	Explain how the study size was arrived at (Page 5)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page 5, 6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 7)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 7)
		(*c*) Explain how missing data were addressed (Page 5)
		(*d*) If applicable, describe analytical methods taking account of sampling strategy (not applicable)
		(*e*) Describe any sensitivity analyses (Page 7)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Page 7, Table 1-3)
		(b) Give reasons for non-participation at each stage (not applicable)
		(c) Consider use of a flow diagram (Figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 7-8, Table 1-3)
		(b) Indicate number of participants with missing data for each variable of interest (Not applicable)
Outcome data	15*	Report numbers of outcome events or summary measures (Page 7-8, Table 3-4)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Page 7-8, Table 4)

		(*b*) Report category boundaries when continuous variables were categorized (Page 5-6)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (Not applicable)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Not applicable)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 8-10)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 9-11)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 9-10)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 10)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 11)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.