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Title Page

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Increased intrahepatic triglyceride content in subjects with metabolically healthy abdominal obesity was associated with excessive risks of prediabetes plus diabetes

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ABSTRACT

Objectives We aimed to evaluate the association of intrahepatic triglyceride (IHTG) content in subjects with metabolically healthy abdominal obesity (MHAO) on risks of prediabetes plus diabetes.

Design Cross-sectional survey.

Setting Lianqian community, the First Affiliated Hospital of Xiamen University, Xiamen, China.

Participants Among 1,523 community-living healthy adults aged 40 years or older with abdominal obesity recruited at baseline, 428 subjects who underwent intrahepatic triglyceride (IHTG) content measurement selected at random chose to participate.

Outcome measures metabolically healthy abdominal obesity (MHAO), metabolically unhealthy abdominal obesity (MUAO), prediabetes, and diabetes.

Results Nonalcoholic fatty liver disease (NAFLD) were diagnosed as 203 (69.1%) in MHAO and 121 (90.3%) in metabolically unhealthy abdominal obesity (MUAO) ($p<0.001$). The prevalence rates of prediabetes plus diabetes were 81.1%, 88.8% and 90.9% across the tertiles of IHTG content ($p=0.037$). Both MUAO (v.s. MHAO) and NAFLD (v.s. Non-NAFLD) were independently associated with increased risks of prediabetes plus diabetes, the adjusted ORs (95%CIs) were 10.90 (3.15-37.69, $p<0.001$) and 3.02 (1.47-6.20, $p=0.003$), respectively. Higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, $p=0.024$). And there was a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes (trend test

p-value =0.011). Stratified analyses showed similar results on the associations of NAFLD and IHTG content with risks of prediabetes plus diabetes for subjects with MHAO but not for those with MUAO.

Conclusions: NAFLD and higher IHTG content were independently associated with increased risks of prediabetes plus diabetes in MHAO subjects. NAFLD or quantity of liver fat should be considered as additional criterion when defining and diagnosing MHO. Screening of NAFLD and intervention to reduce liver fat should be strengthened even for the seemly healthy obese.

Keywords: metabolically healthy obesity; intrahepatic triglyceride; nonalcoholic fatty liver disease; prediabetes; diabetes;

Strengths and limitations of this study

- There was a significantly positive trend of higher prevalence of diabetes plus diabetes with increasing categories of tertiles of IHTG content.
- Both NAFLD and higher IHTG content were independently associated with increased risks of prediabetes plus diabetes in MHAO subjects.
- The current study indicate that quantity of liver fat or NAFLD should be considered as additional criterion when defining and diagnosing MHO.

INTRODUCTION

The global prevalence of diabetes in 2019 was estimated to be 9.3% (463 million people) and would rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045, which has quadrupled during the past three decades and has contributed a heavy public health burden worldwide [1-3]. Obesity has been well documented to contribute a broad array of chronic non-communicable diseases, including diabetes, hypertension, coronary heart disease, chronic kidney disease and certain sites of cancer [4-6]. A subgroup of obese individuals who are devoid of obesity-related metabolic complications, such as diabetes and atherosclerosis, arise the concept of metabolically healthy obese (MHO) [7-9]. However, there is no unique definition and diagnose criteria for MHO by now. For example, some defined MHO when two or fewer of the 4 criteria of metabolism syndrome [10] for those obese subjects while others defined as none of them [11], which made evidence on the association of MHO with diabetes was limited and controversial [12].

Nonalcoholic fatty liver disease (NAFLD) is well documented to be associated with risk of diabetes [13], while NAFLD or excessive fat accumulation in liver which usually occurs simultaneously when obesity happens has not been considered as additional criterion for MHO. Therefore, little evidence is available on the risk of NAFLD or liver fat with diabetes for those with MHO. In the present study with 428 community-living Chinese adults with abdominal obesity, we mainly aimed to evaluate associations of intrahepatic triglyceride (IHTG) content and NAFLD in subjects with metabolically healthy abdominal obesity (MHAO) on risks of prediabetes plus diabetes.

MATERIALS AND METHODS

Study design and subjects

Details on study design and subjects recruitment have been described previously [14,15]. Briefly, 1,523 community-living healthy adults aged 40 years or older with abdominal obesity (waist circumference greater than 90 cm for men and 80 cm for women) living in Lianqian community, Xiamen, China were recruited at baseline of the cohort study in 2011. Of them, a random sample of 428 subjects who underwent intrahepatic triglyceride (IHTG) content measurement was left for the present analysis. This study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Xiamen University (Xiamen, China) and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant.

Measurements

For each subject, face-to-face interview was conducted to collect socio-demographic status, lifestyle habits, present and previous history of health and medications. Subjects were excluded if they drank regularly with alcohol consumption ≥140 g/week for men or ≥70 g/week for women, had cancer, or received current treatment with systemic corticosteroids, biliary obstructive diseases, acute or chronic virus hepatitis, drug-induced liver diseases, total parenteral nutrition, autoimmune hepatitis, Wilson's disease, known hyperthyroidism or hypothyroidism. Subjects underwent weight, height and waist circumference measurements by using a calibrated scale after removing shoes and heavy clothes. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Arterial blood

pressure (BP) was measured with a mercury sphygmomanometer after sitting for at least 15 minutes.

Blood samples were obtained after 12-hour fasting and 75-g oral glucose tolerance test were conducted for each subject. All biochemical measurements were tested in the central laboratory of the First Affiliated Hospital, Xiamen University. Plasma glucose and serum lipid profiles, including triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined on a HITACHI 7450 analyzer (HITACHI, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula. Fasting plasma glucose (FPG) and 2-hour plasma glucose (2-h PG) concentrations were measured by the hexokinase method and HbA1c by the Bio-Rad Variant Hemoglobin A1c assay.

Ultrasonography and definition of non-alcoholic fatty liver disease

Hepatic ultrasonography scanning was performed by an experienced radiologist using GE LOGIQ P5 scanner (GE Healthcare, Milwaukee, USA) with a 4-MHz probe, who was blinded to the subjects' health status. Hepatic steatosis was diagnosed on the basis of characteristic sonographic features, including hepatorenal echo contrast, liver parenchymal brightness, deep beam attenuation, and vessel blurring [16]. The definition of NAFLD was based on hepatic ultrasonography diagnosis of hepatic steatosis without excessive alcohol consumption, viral or autoimmune liver disease.

Intrahepatic triglyceride (IHTG) content measurement

IHTG content was determined by an experienced radiologist using Magnetic resonance spectroscopy (^{1}H MRS, 3.0-T Avanto, Siemens AG, Erlangen, German). Images of a sagittal, coronal and axial cube of a 2 cm^3 volume in the right lobe of liver was acquired. Quantification of the spectra (water and methylene resonances) was performed as described previously [17]. Areas of resonance from water protons and methylene groups in fatty acid chains were obtained with a time-domain non-linear fitting routine by using Syngo MR B15V software (Siemens AG). The percentage of IHTG content was calculated as the ratio of the area under the resonance of peak for methylene groups in fatty acid chains of IHTG and the combined area under the resonance peaks for both methylene groups and water.

Definition of metabolically healthy abdominal obesity

Abdominal obesity was defined as WC ≥90cm for men and 80cm for women [18]. All subjects in the present study were abdominal obesity which was considered as one of the recruitment criterion. Subjects were defined as metabolically healthy abdominal obesity (MHAO) if two or fewer of the following criteria were met: ①systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg; ②fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L); ③TG ≥ 150 mg/dL (1.7 mmol/L); ④HDL cholesterol < 40 mg/dL (1.03 mmol/L) in men and < 50 mg/dL (1.30 mmol/L) in women [19,20]. Otherwise, subjects meeting 3 or more of the criteria were defined as metabolically unhealthy abdominal obesity (MUAO). Therefore, all subjects in the present study were dichotomized as either MHAO or MUAO.

Definitions of diabetes and prediabetes

According to American Diabetes Association (ADA) 2020 criteria, diabetes was defined as (1) a self-reported history of diabetes previously diagnosed by health care professionals; (2) FGP ≥126 mg/dL (7.0mmol/L); (3) 2-hour plasma glucose (2-h PG, OGTT) ≥200 mg/dL (11.1mmol/L); or (4) HbA1c ≥6.5%. Prediabetes were defined as (1) FPG levels between 100 mg/dL (5.6mmol/L) and 125 mg/ dL (6.9mmol/L), (2) 2-h PG levels between 140 mg/dL (7.8mmol/L) and 199 mg/dL (11.0mmol/L), or (3) HbA1c between 5.7% and 6.4% in participants without a prior diabetes diagnosis [21].

Statistical analyses

Data were presented as the mean ± standard deviation for continuous variables or number and percentage for categorical variables. Skewness and kurtosis tests for continuous variables were conducted and found them followed approximation of normal distributions. Differences between subjects categorized by MHAO and tertiles of IHTG content were analyzed using one-way ANOVA for continuous variables and chi-square test for categorical variables. Bar graphs showing prevalence rates of diabetes, prediabetes and normal glucose test (NGT) were made by MHAO (v.s. MUAO) and tertiles of IHTG content.

Multivariable logistic regression models were used to calculate the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of abdominal obesity (MUAO v.s. MHAO), NAFLD (yes v.s. no) and IHTG content (both the originally continuous values and the tertiles categories) for prediabetes plus diabetes with adjustment for potential confounders (including age, sex,

educational level, smoking and drinking habits, regular physical exercise, BMI, systolic and diastolic BP, triglyceride, total cholesterol, HDL- and LDL-cholesterol and serum uric acid). And multivariable logistic regression analyses stratified by MHAO and MUAO groups were further conducted. All p-values were two-sided and p-value <0.05 was considered statistically significant. All statistical analyses were performed using Stata14.0 (StatCorp, College Station, TX).

Patient and public involvement

There were no funds or time allocated for patient and public involvement.

RESULTS

Prevalence of diabetes and prediabetes stratified by MHAO and tertiles of IHTG content

Among the 428 subjects with abdominal obesity, MHAO and MUAO were identified on 294 (68.7%) and 134 (31.3%) subjects. Of them, 46 (10.8%), 326 (76.2%) and 56 (13.1%) were diagnosed as diabetes, prediabetes and normal glucose tolerance (NGT), respectively. There was a significantly positive trend between increasing tertiles of IHTG content and higher prevalence of prediabetes plus diabetes (81.1%, 88.8% and 90.9% across the tertiles of IHTG content (p=0.037)). Figure 1 showed the prevalence rates of diabetes and prediabetes across the tertiles of IHTG content in MHAO subjects were 7.1% and 67.3%, 10.2% and 74.5%, 10.2% and 77.6% for the Tertile 1, Tertile 2 and Tertile 3, respectively (p-value>0.05). But there was a significantly positive trend of higher prevalence of diabetes plus diabetes with increasing categories of tertiles of IHTG content (p=0.039). Figure 2 showed the prevalence rates of diabetes and prediabetes across the tertiles of IHTG content in MUAO subjects were 8.9% and 86.7%, 8.9% and 88.9%, 25.0% and 72.7% for Tertile 1, Tertile 2 and Tertile 3, respectively. Table 1 also showed MUAO subjects had significantly higher prevalence of prediabetes and prediabetes plus diabetes than MHAO subjects (both p-values<0.05).

Demographic and clinical characteristics stratified MHAO and tertiles of IHTG content

For all the 428 subjects, the means (±SD) of age were 53.6 (±6.5) years for women (n=319, 74.5%) and 53.2 (±7.1) years for men (n=109, 25.5%) (p=0.592). Table 1 showed differences of demographics, life style habits and clinical characteristics stratified by MHAO and tertiles

of IHTG content. For 294 MHAO subjects, with increasing categories of the tertiles of IHTG content (from tertile 1, tertile 2 to tertile 3), subjects were more likely to be male and had significantly higher levels of indices of obesity (BMI, waist circumference), diastolic BP, triglyceride, HbA1c, serum uric acid as well as higher prevalence of NAFLD and significantly lower level of HDL-C. As for 134 MUAO subjects, increasing categories of the tertiles of IHTG content were significantly related to higher prevalence of NAFLD and serum uric acid levels. Furthermore, Table 1 showed that, compared to subjects with MHAO, those with MUAO had significantly increased age, IHTG content, prevalence of NAFLD, systolic and diastolic BP, triglyceride, total cholesterol, FPG, 2-h PG, HbA1c, serum uric acid and significantly lower level of HDL-C.

Associations of MHAO, NAFLD and IHTG content with prediabetes plus diabetes for all subjects

Table 2 showed that, for all subjects, both MUAO (v.s. MHAO) and NAFLD (yes v.s. no) were independently associated with increased risk of prediabetes plus diabetes, and the adjusted ORs (95%CIs) were 10.90 (3.15-37.69, p<0.001) and 3.02 (1.47-6.20, p=0.003), respectively. Higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, p=0.024). With the tertile 1 of IHTG content as the reference, the tertile 3 showed significantly higher risk of prediabetes plus diabetes (adjusted OR (95%CI): 3.13 (1.28-7.61), p=0.012). And there was a significantly positive trend of increasing categories of IHTG content tertiles with excessive risk of prediabetes plus diabetes (trend test: p=0.011).

There was no significant interaction between MHAO with either NAFLD or tertiles of IHTG content for risk of prediabetes plus diabetes (both p-values>0.05).

Stratified analyses on associations of NAFLD and IHTG content with prediabetes plus diabetes by MHAO & MUAO

Multivariable logistic regression analyses stratified by MHAO and MUAO separately were conducted. For MHAO subjects, NAFLD was independently associated with increased risk of prediabetes plus diabetes (adjusted OR (95%CI): 2.65 (1.25-5.60), p=0.011). Per SD increase of IHTG content was marginally associated with excessive risk of prediabetes plus diabetes with the adjusted OR (95%CI) of 1.55 (1.00-2.40, p=0.051). Compared with the tertile 1 of IHTG content, both the tertile 2 and tertile 3 groups showed significantly increased risks of prediabetes plus diabetes with the adjusted ORs (95%CI) of 2.31 (1.03-5.17, p=0.042) and 2.81 (1.14-6.90, p=0.024), respectively. And there was also a significantly positive trend between increasing categories of IHTG content tertiles and excessive risk of prediabetes plus diabetes (trend test: p=0.021). For MUAO subjects, neither NAFLD nor IHTG content was found to be significantly associated with risk of prediabetes plus diabetes.

DISCUSSION

In the present study of 428 subjects with abdominal obesity, 294 (68.7%) and 134 (31.3%) were identified as MHAO and MUAO, respectively. For all subjects, the prevalence rates of prediabetes plus diabetes were 81.1%, 88.8% and 90.9% across the tertiles of IHTG content (p=0.037). Both MUAO (v.s. MHAO) and NAFLD (v.s. Non-NAFLD) were independently associated with increased risks of prediabetes plus diabetes. Furthermore, higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, p=0.024), and there was a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes. Stratified analyses showed similar results for subjects with MHAO but not for those with MUAO.

The concept of MHO has been established for a subgroup of obese subjects who do not exhibit metabolic and cardiovascular complications at a given time point, such as diabetes and atherosclerosis, for a few decades [22,23]. Compared to subjects with MUO, those with MHO are characterized by lower liver and visceral fat, higher subcutaneous leg fat, greater cardiorespiratory fitness, physical activity and insulin sensitivity, lower levels of inflammation, and normal adipose tissue function [24]. However it could be debated whether MHO predicts the risk of diabetes compared with metabolically healthy normal weight or MUO. Hinnouho GM et al, based on the Whitehall II cohort study, found a significantly decreased risk for MHO compared with metabolically unhealthy obesity (MUO) (HR=1.98 (MUO v.s. MHO), 95% CI: 1.39–2.83) [25]. The present study found similar results that

MUAO was significantly associated with increased risk of prediabetes plus diabetes compared with MHAO but with a much higher adjusted OR(95%CI) (10.90 (3.15-37.69)). Hinnouho GM et al and others further found that MHO showed a significant increased risk of T2DM incidence compared with metabolically healthy normal weight [25-27]. We cannot evaluate the risk of MHAO on diabetes compared with metabolically healthy normal weight since all the subjects in the present study were central obese and none of them could be classified as metabolically healthy or unhealthy normal weight. And because we had a relatively small sample size, we might find the adjusted OR was much higher than those from other [25-27].

MHO is generally characterized by lower liver fat; but little evidence is available on differences of NAFLD prevalence or liver fat content between MHO and MUO. In the present study, we found subjects with MUO, compared to those with MHO, showed significantly higher prevalence of NAFLD (90.3% v.s. 69.1%) and IHTG content (16.3±9.5 v.s. 12.3±9.5%) (both p-values<0.001). Our findings indicated that, even for those with MHO, presence of NAFLD and IHTG content are still common and high. Meanwhile MHO is commonly identified based on the presence of obesity and metabolic syndrome, neither NAFLD nor liver fat content has been considered as an additional criterion when defining and diagnosing MHO. Therefore, our findings implied that screening of NAFLD and intervention to reduce IHTG content for those seemly healthy obese should be strengthened. NAFLD has been well documented to predict diabetes incidence [13], and our previous findings showed that NAFLD was significantly associated with increased risk of T2DM

prevalence [28]. The present study expand the positive association of NAFLD to risk of prediabetes plus diabetes for all subjects as well as for those with MHAO with the adjusted ORs (95%CI) of 3.02 (1.47-6.20) and 2.65 (1.25-5.60) (both p-values<0.05), resspectively. NAFLD has been generally diagnosed by hepatic ultrasonography scanning, which is considered to be unreliable and difficult to use in obese subjects [29,30]. Therefore, we conducted IHTG content measurement by using magnetic resonance spectroscopy to quantify more precisely the extent of liver fat in these abdominal obese subjects. And we found that IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, p=0.024). Moreover, we found a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes. Our results on the association between IHTG content and risks of prediabetes plus diabetes might account for possibly a novel finding for the present study, since little evidence was available on association between the relatively precise quantity of liver fat and risk of diabetes [31,32].

We further conducted stratified analyses on the associations of IHTG content with risk of prediabetes plus diabetes for subjects with MHAO and MUAO separately. For those with MHAO, the association of IHTG content with risk of prediabetes plus diabetes was marginally significant, and the adjusted OR (95%CI) of per SD increase of IHTG content was 1.55 (1.00-2.40, p=0.051). With the first tertile of IHTG content as the reference group, the adjusted ORs (95%CI) of risks of prediabetes plus diabetes for the 2nd and 3rd tertiles were 2.31 (1.03-5.17) and 2.81 (1.14-6.90) (both p-values<0.05), respectively. The positive trend

between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes was also statistically significant for the subgroup with MHAO (trend test p-value<0.05). Our findings implied that increased intrahepatic triglyceride content was associated with excessive risk of prediabetes and diabetes even for MHO subjects. To the best of our knowledge, we were probably the first to find the positive associations of IHTG content with risks of diabetes and prediabetes for MHAO subjects. The reason for non-significant results for MUAO subgroups may be due to the relatively small sample size of subjects with MUAO (n=134).

NAFLD and liver fat quantity has not been currently considered in the definitions and diagnose criteria of MHO [24], although liver is one of the main parts of fat accumulation when obesity occurs. The present study found that around 69% of subjects with MHAO were diagnosed as NAFLD. Most importantly, even for these apparently healthy obese individuals, NAFLD and higher IHTG content were both significantly associated with increased risks of prediabetes plus diabetes. Therefore, our findings implied that the current criteria of MHO may not be appropriate, and NAFLD or quantity of liver fat should be considered as additional criterion when defining and diagnosing MHO if more evidence could prove our findings in future, especially from the prospective cohort studies with larger sample sizes.

A few limitations of the present study should be recognized when generalizing our findings to other populations. Firstly, all subjects were abdominally obese and were not randomly sampled from their living communities; therefore we could not assess the effect of MHAO as compared with metabolically healthy non-obesity and we might also under-estimate the true

associations of MHAO as compared with MUAO on risks of prediabetes plus diabetes. Secondly, the present analyses were based on the baseline information of our ongoing cohort study, therefore we cannot determine the temporal sequence among MHAO and prediabetes plus diabetes. Thirdly, our sample size was small, especially for the MUAO subgroup and we may not have enough power to determine their true associations. On the other hand, we still have some strengths in the present study. For example, we used IHTG content by magnetic resonance spectroscopy, which was relatively more precise measurement of liver fat. And we were probably the first to find the positive associations of IHTG content with risks of diabetes and prediabetes, especially for subjects with MHAO.

CONCLUSIONS

NAFLD were diagnosed in 69% of MHAO and 90% of MUAO subjects, and the prevalence rates of prediabetes plus diabetes were linearly increased across the tertiles of IHTG content. NAFLD and higher IHTG content were independently associated with increased risks of prediabetes plus diabetes for all subjects as well as for the MHAO subgroups. Therefore, our findings imply that NAFLD or quantity of liver fat should be considered as additional criterion when defining and diagnosing MHO. Furthermore, screening of NAFLD and intervention to reduce liver fat should be strengthened even for the seemly healthy obese subjects.

Declarations

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Author contributions

Q.X., J.Z., S.W., N.C. and Z.L. performed the statistical analysis and wrote the manuscript; M.L. and Y.L. participated in the data collection; F.L. and W.Z. contributed to discussion; C.L., N.C., S.W. and J.Z. participated in the design of the study and edited the manuscript. C.L. and Z.L. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest statement

No potential conflict of interest relevant to this article is declared.

Ethical approval

This study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Xiamen University (Xiamen, China) (number/ID of the obtained ethics approval:

2011YLS-013) and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figure Legends

Figure 1. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MHAO subjects.

Figure 2. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MUAO subjects.

Table Legends

Table 1. Demographic, lifestyle and clinical characteristics of 428 subjects stratified by MHAO and tertiles of IHTG content.

Table 2. Adjusted odds ratios (ORs) with associated 95% confidence interval (CI) of MUAO, NAFLD and IHTG content for prediabetes plus diabetes.

Table 1. Demographic, lifestyle and clinical characteristics of 428 subjects stratified by MHAO and tertiles of IHTG content.

Variables	MHAO (n=294, 68.7%)				MUAO (n=134, 31.3%)				P-value §
	Tertile 1	Tertile 2	Tertile 3	P-value	Tertile 1	Tertile 2	Tertile 3	P-value	
Demographics & life style									
N (%)	98 (33.3%)	98 (33.3%)	98 (33.3%)		45 (33.6%)	45 (33.6%)	44 (32.8%)		
Male gender (n, %)	12 (12.4%)	27 (27.6%)	30 (30.6%)	0.005*	11 (24.4%)	17 (38.8%)	12 (27.3%)	0.347	0.160
Age (years)	52.8±6.5	53.6±6.6	52.4±6.8	0.422	54.8±6.1	54.1±7.5	55.0±6.0	0.821	0.014*
Low educational attainment, (n, %)	61 (62.2%)	48 (49.0%)	51 (52.0%)	0.241	30 (66.7%)	25 (55.6%)	27 (61.4%)	0.110	0.090
Ever smoking (n, %)	16 (16.3%)	25 (25.5%)	23 (23.5%)	0.262	9 (20.0%)	11 (24.4%)	11 (25.0%)	0.828	0.753
Ever drinking (n, %)	8 (8.2%)	13 (13.3%)	15 (15.3%)	0.291	2 (4.4%)	6 (13.3%)	4 (9.1%)	0.216	0.317
Regular physical exercise (n, %)	34 (34.7%)	42 (42.9%)	32 (32.7%)	0.292	13 (28.9%)	17 (37.8%)	10 (22.7%)	0.296	0.165
Clinical characteristics									
IHTG content (%)	4.10±1.63	9.64±1.97	23.25±8.47	<0.001†	6.86±2.44	14.54±2.91	27.68±6.21	<0.001†	<0.001†
NAFLD (n, %)	34 (34.7%)	75 (76.5%)	94 (95.9%)	<0.001†	35 (77.8%)	42 (93.3%)	44 (100.0%)	<0.001†	<0.001†
BMI (kg/m^2)	26.2±2.5	27.2±2.5	28.3±2.7	<0.001†	27.3±2.5	27.7±3.5	28.1±2.8	0.485	0.121
Waist circumference (cm)	90.7±5.1	93.5±6.1	96.0±7.1	<0.001†	94.0±5.3	93.7±7.8	95.3±6.8	0.518	0.158
Systolic blood pressure (mmHg)	125.6±17.3	130.6±17.5	129.5±15.7	0.095	139.8±12.4	140.9±15.3	143.4±12.6	0.451	<0.001†
Diastolic blood pressure (mmHg)	74.0±10.0	77.8±9.8	78.4±9.7	0.004*	82.7±10.7	83.7±9.4	85.2±8.3	0.482	<0.001†
Triglyceride (mmol/L)	1.22±0.57	1.56±0.82	1.89±0.98	<0.001†	2.53±1.26	2.32±1.90	2.84±1.52	0.062	<0.001†
Total cholesterol (mmol/L)	5.80±1.03	5.81±0.95	5.95±1.05	0.487	6.18±1.43	6.13±1.27	6.02±0.94	0.823	0.024*
HDL-cholesterol (mmol/L)	1.50±0.26	1.39±0.29	1.37±0.25	0.003*	1.20±0.19	1.14±0.22	1.18±0.20	0.401	<0.001†
LDL-cholesterol (mmol/L)	3.75±0.95	3.72±0.79	3.75±1.01	0.973	3.83±1.30	3.50±1.41	3.56±0.84	0.391	0.297

Blood uric acid (μmol/L)	322.9±71.5	359.8±83.6	378.2±98.1	<0.001†	348.3±84.2	391.6±90.9	426.0±108.7	<0.001†	0.001*
Fasting plasma glucose (mmol/L)	5.56±0.46	5.55±0.54	5.54±0.51	0.967	5.85±0.44	5.98±0.45	5.94±0.42	0.338	<0.001†
2-h PG (OGTT, mmol/L)	7.42±2.52	7.51±1.86	7.87±1.88	0.285	8.04±1.91	8.09±1.72	8.87±2.13	0.082	<0.001†
HbA1c (%)	5.86±0.29	5.93±0.36	5.97±0.29	0.041*	6.01±0.32	6.00±0.38	6.04±0.36	0.882	0.005*
Diabetes (n, %)	7 (7.1%)	10 (10.2%)	10 (10.2%)	0.693	4 (8.9%)	4 (8.9%)	11 (25.0%)	0.043*	0.122
Prediabetes (n, %)	66 (67.3%)	73 (74.5%)	76 (77.6%)	0.255	39 (86.7%)	40 (88.9%)	32 (72.7%)	0.091	0.039*
Prediabetes plus diabetes (n, %)	73 (74.5%)	83 (84.7%)	86 (87.8%)	0.039*	43 (95.6%)	44 (97.8%)	43 (97.7%)	0.779	<0.001†

* p<0.05, †p<0.001, § P-value for difference between MHAO and MUAO.

All percentages are column percentage; except for percentages, all values are mean±s.d. .

Abbreviations: 2-h PG, 2-hour plasma glucose; BMI, body mass index; HDL, high-density lipoprotein; IHTG, intrahepatic triglyceride; LDL, low-density lipoprotein cholesterol; MHAO, metabolically healthy abdominal obesity; MUAO, metabolically unhealthy abdominal obesity; NAFLD,non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test.

Table 2. Adjusted odds ratios (ORs) with associated 95% confidence interval (CI) of MUAO, NAFLD and IHTG content for prediabetes plus diabetes.

	Prediabetes plus Diabetes		
Variables	OR	95% CI	P-value
	All subjects		
MUAO v.s. MHAO	10.90	3.15 - 37.69	<0.001*
NAFLD v.s. Non-NAFLD	3.02	1.47 - 6.20	0.003*
IHTG content (%) †	1.62	1.07 - 2.46	0.024*
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	1.81	0.86 - 3.81	0.117
Tertile 3	3.13	1.28 - 7.61	0.012*
Trend test			0.011*
Interaction test			
MUAO*NAFLD			0.956
MUAO*Tertiles of IHTG			0.869
	MHAO subjects		
NAFLD v.s. Non-NAFLD	2.65	1.25 - 5.60	0.011*
IHTG content (%) †	1.55	1.00 - 2.40	0.051
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	2.31	1.03 - 5.17	0.042*
Tertile 3	2.81	1.14 - 6.90	0.024*
Trend test			0.021*
	MUAO subjects		
NAFLD v.s. Non-NAFLD	4.77	0.07 - 327.48	0.469
IHTG content (%) †	0.81	0.13 - 5.26	0.830
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	3.22	0.24 - 43.54	0.378
Tertile 3	1.90	0.15 - 23.69	0.620
Trend test			0.558

*p<0.05

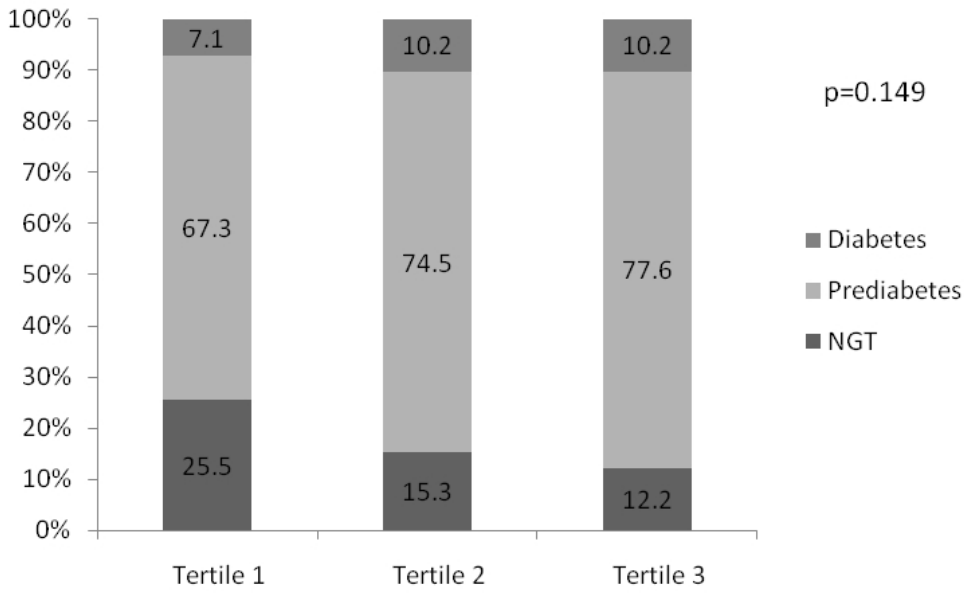
† OR and 95%CI was expressed by per SD increase of IHTG content.

‡ OR and 95%CI was expressed by the first quartile of IHTG content as the reference.

Abbreviations: CI, confidence interval; IHTG, intrahepatic triglyceride; MHAO, metabolically healthy abdominal obesity; MUAO, metabolically unhealthy abdominal obesity; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio;

OR was adjusted for age, sex, educational level, ever smoking, ever drinking, physical

activity, BMI, systolic and diastolic BP, triglyceride, total cholesterol, HDL- and LDL-cholesterol and serum uric acid.



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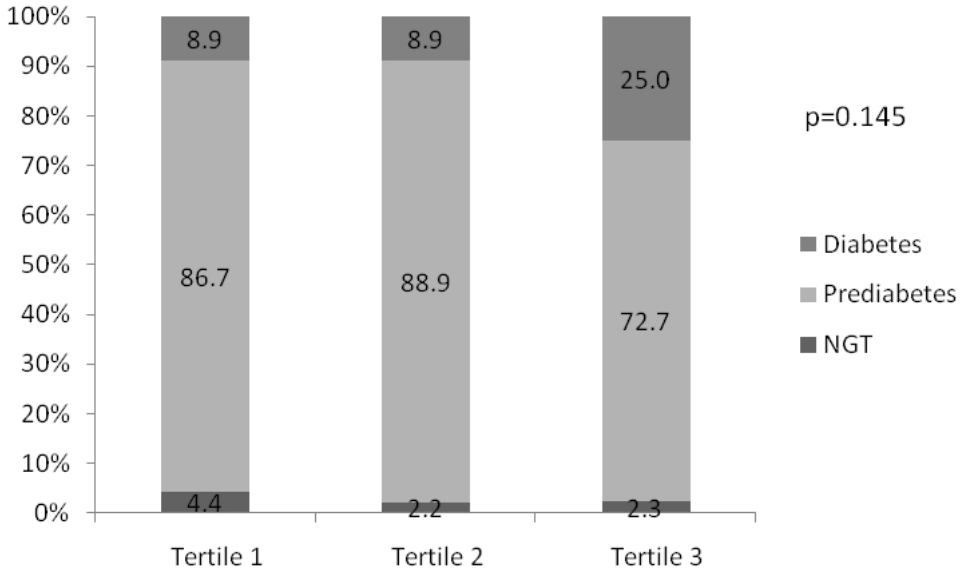

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
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	*7-9*
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
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	12
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	12-13
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	13-14

		(*b*) Report category boundaries when continuous variables were categorized	13-14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-18
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	19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19
Generalisability	21	Discuss the generalisability (external validity) of the study results	19
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	3

*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 between intrahepatic triglyceride content in subjects with metabolically healthy abdominal obesity and risks of prediabetes plus diabetes: an observational study.

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Title Page

Manuscript Type: Original Article

Association between intrahepatic triglyceride content in subjects with metabolically healthy abdominal obesity and risks of prediabetes plus diabetes: an observational study

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ABSTRACT

Objective We aimed to evaluate the association of intrahepatic triglyceride (IHTG) content in subjects with metabolically healthy abdominal obesity (MHAO) on risks of prediabetes plus diabetes.

Design Cross-sectional survey.

Setting Lianqian community, the First Affiliated Hospital of Xiamen University, Xiamen, China.

Participants Among 1,523 community-living healthy adults aged 40 years or older with abdominal obesity recruited at baseline, 428 subjects who underwent intrahepatic triglyceride (IHTG) content measurement selected at random chose to participate.

Outcome measures metabolically healthy abdominal obesity (MHAO), metabolically unhealthy abdominal obesity (MUAO), prediabetes, and diabetes.

Results Nonalcoholic fatty liver disease (NAFLD) were diagnosed as 203 (69.1%) in MHAO and 121 (90.3%) in metabolically unhealthy abdominal obesity (MUAO) ($p<0.001$). The prevalence rates of prediabetes plus diabetes were 81.1%, 88.8% and 90.9% across the tertiles of IHTG content ($p=0.037$). Both MUAO (v.s. MHAO) and NAFLD (v.s. Non-NAFLD) were independently associated with increased risks of prediabetes plus diabetes, the adjusted ORs (95%CIs) were 10.90 (3.15-37.69, $p<0.001$) and 3.02 (1.47-6.20, $p=0.003$), respectively. Higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, $p=0.024$). And there was a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes (trend test

p-value =0.011). Stratified analyses showed similar results on the associations of NAFLD and IHTG content with risks of prediabetes plus diabetes for subjects with MHAO but not for those with MUAO.

Conclusions: NAFLD and higher IHTG content were independently associated with increased risks of prediabetes plus diabetes in MHAO subjects. NAFLD or quantity of liver fat should be considered as additional criterion when defining and diagnosing MHO. Screening of NAFLD and intervention to reduce liver fat should be strengthened even for the seemly metabolically healthy obese.

Keywords: metabolically healthy obesity; intrahepatic triglyceride; nonalcoholic fatty liver disease; prediabetes; diabetes;

Strengths and limitations of this study

- IHTG content was determined using magnetic resonance spectroscopy, which was relatively quantitative measurement of liver fat.
- Both NAFLD and higher IHTG content were independently associated with increased risks of prediabetes plus diabetes in MHAO subjects.
- Quantity of liver fat or NAFLD should be considered as additional criterion when defining and diagnosing MHO, and screening of NAFLD and intervention to reduce liver fat should be strengthened even for the seemly metabolically healthy obese subjects.

INTRODUCTION

The prevalence of diabetes has quadrupled during the past three decades with an estimated prevalence of 9.3% (463 million people) in 2019 and it is expected to rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [1-3]. Obesity has been well documented to be a risk factor for a broad array of chronic non-communicable diseases, including diabetes, hypertension, coronary heart disease, chronic kidney disease and certain sites of cancer [4-6]. A subgroup of obese individuals who are devoid of obesity-related metabolic complications, such as diabetes and atherosclerosis, arise the concept of metabolically healthy obese (MHO) [7-9]. However, there is no unique definition and diagnose criteria for MHO by now. For example, some defined MHO when two or fewer of the 4 criteria of metabolism syndrome [10] for those obese subjects while others defined as none of them [11], which made evidence on the association of MHO with diabetes was limited and controversial [12].

Nonalcoholic fatty liver disease (NAFLD) is well documented to be associated with risk of diabetes [13], however NAFLD has not been considered as additional criterion for MHO although it usually occurs simultaneously when obesity happens. Therefore, little evidence is available on the risk of NAFLD or liver fat with diabetes for those with MHO. In the present study with 428 community-living Chinese adults with abdominal obesity, we mainly aimed to evaluate associations of intrahepatic triglyceride (IHTG) content and NAFLD in subjects with metabolically healthy abdominal obesity (MHAO) on risks of prediabetes plus diabetes.

METHODS

Study design and subjects

Details on study design and subjects recruitment have been described previously [14,15]. Briefly, 1,523 community-living healthy adults aged 40 years or older with abdominal obesity (waist circumference greater than 90 cm for men and 80 cm for women) living in Lianqian community, Xiamen, China were recruited at baseline of the cohort study in 2011. Of them, 92 (6%) who had incomplete data on clinical and biochemistry measurements were excluded, and a random sample of 428 subjects who underwent intrahepatic triglyceride (IHTG) content measurement was left for the present analysis (Figure 1). Of the 428 study subjects, 319 (74.5%) were female with the mean age of 53.6±6.5 years old, 109 (25.5%) were male with the mean age of 53.2±7.1 years old, and there was no significant difference in age between male and female subjects (p=0.592). This study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Xiamen University (Xiamen, China) and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant.

Measurements

For each subject, face-to-face interview was conducted to collect socio-demographic status, lifestyle habits, present and previous history of health and medications. Subjects were excluded if they drank regularly with alcohol consumption ≥140 g/week for men or ≥70 g/week for women, had cancer, or received current treatment with systemic corticosteroids, biliary obstructive diseases, acute or chronic virus hepatitis, drug-induced liver diseases, total

parenteral nutrition, autoimmune hepatitis, Wilson's disease, known hyperthyroidism or hypothyroidism. Subjects underwent weight, height and waist circumference measurements by using a calibrated scale after removing shoes and heavy clothes. Waist circumference was measured at the midpoint between the inferior costal margin and the superior border of the iliac crest on the midaxillary line. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Arterial blood pressure (BP) was measured with a mercury sphygmomanometer after sitting for at least 15 minutes.

Blood samples were obtained after 12-hour fasting and 75-g oral glucose tolerance test were conducted for each subject. All biochemical measurements were tested in the central laboratory of the First Affiliated Hospital, Xiamen University. Plasma glucose and serum lipid profiles, including triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined on a HITACHI 7450 analyzer (HITACHI, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula. Fasting plasma glucose (FPG) and 2-hour plasma glucose (2-h PG) concentrations were measured by the hexokinase method and HbA1c by the Bio-Rad Variant Hemoglobin A1c assay.

Ultrasonography and definition of non-alcoholic fatty liver disease

Hepatic ultrasonography scanning was performed by an experienced radiologist using GE LOGIQ P5 scanner (GE Healthcare, Milwaukee, USA) with a 4-MHz probe, who was blinded to the subjects' health status. Hepatic steatosis was diagnosed on the basis of

characteristic sonographic features, including hepatorenal echo contrast, liver parenchymal brightness, deep beam attenuation, and vessel blurring [16]. The definition of NAFLD was based on hepatic ultrasonography diagnosis of hepatic steatosis without excessive alcohol consumption, viral or autoimmune liver disease.

Intrahepatic triglyceride (IHTG) content measurement

IHTG content was determined by an experienced radiologist using Magnetic resonance spectroscopy (^{1}H MRS, 3.0-T Avanto, Siemens AG, Erlangen, German). Images of a sagittal, coronal and axial cube of a 2 cm^3 volume in the right lobe of liver was acquired. Quantification of the spectra (water and methylene resonances) was performed as described previously [17]. Areas of resonance from water protons and methylene groups in fatty acid chains were obtained with a time-domain non-linear fitting routine by using Syngo MR B15V software (Siemens AG). The percentage of IHTG content was calculated as the ratio of the area under the resonance of peak for methylene groups in fatty acid chains of IHTG and the combined area under the resonance peaks for both methylene groups and water.

Definition of metabolically healthy abdominal obesity

Abdominal obesity was defined as WC ≥90cm for men and 80cm for women [18]. All subjects in the present study were abdominal obesity which was considered as one of the recruitment criteria. Subjects were defined as metabolically healthy abdominal obesity (MHAO) if two or fewer of the following criteria were met: ①systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg; ②fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L); ③TG ≥ 150

mg/dL (1.7 mmol/L); ④HDL cholesterol < 40 mg/dL (1.03 mmol/L) in men and < 50 mg/dL (1.30 mmol/L) in women [19,20]. Otherwise, subjects meeting 3 or more of the criteria were defined as metabolically unhealthy abdominal obesity (MUAO). Therefore, all subjects in the present study were dichotomized as either MHAO or MUAO.

Definitions of diabetes and prediabetes

According to American Diabetes Association (ADA) 2020 criteria, diabetes was defined as (1) a self-reported history of diabetes previously diagnosed by health care professionals; (2) FGP ≥126 mg/dL (7.0mmol/L); (3) 2-hour plasma glucose (2-h PG, OGTT) ≥200 mg/dL (11.1mmol/L); or (4) HbA1c ≥6.5%. Prediabetes was defined as (1) FPG levels between 100 mg/dL (5.6mmol/L) and 125 mg/ dL (6.9mmol/L), (2) 2-h PG levels between 140 mg/dL (7.8mmol/L) and 199 mg/dL (11.0mmol/L), or (3) HbA1c between 5.7% and 6.4% in participants without a prior diabetes diagnosis [21].

Statistical analyses

Data were presented as the mean ± standard deviation for continuous variables or number and percentage for categorical variables. Skewness and kurtosis tests for continuous variables were conducted and found them followed approximation of normal distributions. Differences between subjects categorized by MHAO and tertiles of IHTG content were analyzed using one-way ANOVA for continuous variables and chi-square test for categorical variables. Bar graphs showing prevalence rates of diabetes, prediabetes and normal glucose test (NGT) were made by MHAO (v.s. MUAO) and tertiles of IHTG content.

Multivariable logistic regression models were used to calculate the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of abdominal obesity (MUAO v.s. MHAO), NAFLD (yes v.s. no) and IHTG content (both the originally continuous values and the tertiles categories) for prediabetes plus diabetes with adjustment for potential confounders (including age, sex, educational level, smoking and drinking habits, regular physical exercise, BMI, systolic and diastolic BP, triglyceride, total cholesterol, HDL- and LDL-cholesterol and serum uric acid). And multivariable logistic regression analyses stratified by MHAO and MUAO groups were further conducted. All p-values were two-sided and p-value <0.05 was considered statistically significant. All statistical analyses were performed using Stata14.0 (StatCorp, College Station, TX).

Patient and public involvement

There were no funds or time allocated for patient and public involvement.

RESULTS

Prevalence of diabetes and prediabetes stratified by MHAO and tertiles of IHTG content

Among the 428 subjects with abdominal obesity, MHAO and MUAO were identified on 294 (68.7%) and 134 (31.3%) subjects. Of them, 46 (10.8%), 326 (76.2%) and 56 (13.1%) were diagnosed as diabetes, prediabetes and normal glucose tolerance (NGT), respectively. There was a significantly positive trend between increasing tertiles of IHTG content and higher prevalence of prediabetes plus diabetes (81.1%, 88.8% and 90.9% across the tertiles of IHTG content (p=0.037)). Figure 2A showed the prevalence rates of diabetes and prediabetes across the tertiles of IHTG content in MHAO subjects were 7.1% and 67.3%, 10.2% and 74.5%, 10.2% and 77.6% for the Tertile 1, Tertile 2 and Tertile 3, respectively (p-value>0.05). But there was a significantly positive trend of higher prevalence of diabetes plus diabetes with increasing categories of tertiles of IHTG content (p=0.039). Figure 2B showed the prevalence rates of diabetes and prediabetes across the tertiles of IHTG content in MUAO subjects were 8.9% and 86.7%, 8.9% and 88.9%, 25.0% and 72.7% for Tertile 1, Tertile 2 and Tertile 3, respectively. Table 1 also showed MUAO subjects had significantly higher prevalence of prediabetes and prediabetes plus diabetes than MHAO subjects (both p-values<0.05).

Demographic and clinical characteristics stratified MHAO and tertiles of IHTG content

For all the 428 subjects, the means (±SD) of age were 53.6 (±6.5) years for women (n=319, 74.5%) and 53.2 (±7.1) years for men (n=109, 25.5%) (p=0.592). Table 1 showed differences of demographics, life style habits and clinical characteristics stratified by MHAO and tertiles

of IHTG content. For 294 MHAO subjects, with increasing categories of the tertiles of IHTG content (from tertile 1, tertile 2 to tertile 3), subjects were more likely to be male and had significantly higher levels of indices of obesity (BMI, waist circumference), diastolic BP, triglyceride, HbA1c, serum uric acid as well as higher prevalence of NAFLD and significantly lower level of HDL-C. As for 134 MUAO subjects, increasing categories of the tertiles of IHTG content were significantly related to higher prevalence of NAFLD and serum uric acid levels. Furthermore, Table 1 showed that, compared to subjects with MHAO, those with MUAO had significantly increased age, IHTG content, prevalence of NAFLD, systolic and diastolic BP, triglyceride, total cholesterol, FPG, 2-h PG, HbA1c, serum uric acid and significantly lower level of HDL-C.

Associations of MHAO, NAFLD and IHTG content with prediabetes plus diabetes for all subjects

Table 2 showed that, for all subjects, both MUAO (v.s. MHAO) and NAFLD (yes v.s. no) were independently associated with increased risk of prediabetes plus diabetes, and the adjusted ORs (95%CIs) were 10.90 (3.15-37.69, p<0.001) and 3.02 (1.47-6.20, p=0.003), respectively. Higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, p=0.024). With the tertile 1 of IHTG content as the reference, the tertile 3 showed significantly higher risk of prediabetes plus diabetes (adjusted OR (95%CI): 3.13 (1.28-7.61), p=0.012). And there was a significantly positive trend of increasing categories of IHTG content tertiles with excessive risk of prediabetes plus diabetes (trend test: p=0.011).

There was no significant interaction between MHAO with either NAFLD or tertiles of IHTG content for risk of prediabetes plus diabetes (both p-values>0.05).

Stratified analyses on associations of NAFLD and IHTG content with prediabetes plus diabetes by MHAO & MUAO

Multivariable logistic regression analyses stratified by MHAO and MUAO separately were conducted. For MHAO subjects, NAFLD was independently associated with increased risk of prediabetes plus diabetes (adjusted OR (95%CI): 2.65 (1.25-5.60), p=0.011). Per SD increase of IHTG content was marginally associated with excessive risk of prediabetes plus diabetes with the adjusted OR (95%CI) of 1.55 (1.00-2.40, p=0.051). Compared with the tertile 1 of IHTG content, both the tertile 2 and tertile 3 groups showed significantly increased risks of prediabetes plus diabetes with the adjusted ORs (95%CI) of 2.31 (1.03-5.17, p=0.042) and 2.81 (1.14-6.90, p=0.024), respectively. And there was also a significantly positive trend between increasing categories of IHTG content tertiles and excessive risk of prediabetes plus diabetes (trend test: p=0.021). For MUAO subjects, neither NAFLD nor IHTG content was found to be significantly associated with risk of prediabetes plus diabetes.

DISCUSSION

In the present study of 428 subjects with abdominal obesity, 294 (68.7%) and 134 (31.3%) were identified as MHAO and MUAO, respectively. Both MUAO (v.s. MHAO) and NAFLD (v.s. Non-NAFLD) were independently associated with increased risks of prediabetes plus diabetes. Furthermore, higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes, and there was a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes. Stratified analyses showed similar results for subjects with MHAO but not for those with MUAO.

The concept of MHO has been established for a subgroup of obese subjects who do not exhibit metabolic and cardiovascular complications at a given time point, such as diabetes and atherosclerosis, for a few decades [22,23]. Compared to subjects with MUO, those with MHO are characterized by lower liver and visceral fat, higher subcutaneous leg fat, greater cardiorespiratory fitness, physical activity and insulin sensitivity, lower levels of inflammation, and normal adipose tissue function [24]. However, it could be debated whether MHO predicts the risk of diabetes compared with metabolically healthy normal weight or MUO. Hinnouho GM et al, based on the Whitehall II cohort study, found a significantly decreased risk of diabetes for MHO compared with metabolically unhealthy obesity (MUO) (HR=1.98 (MUO v.s. MHO), 95% CI: 1.39–2.83) [25]. The present study found similar results that MUAO was significantly associated with increased risk of prediabetes plus diabetes compared with MHAO but with a much higher adjusted OR(95%CI) (10.90 (3.15-37.69)). Hinnouho GM et al and others further found that MHO showed a significant

increased risk of T2DM incidence compared with metabolically healthy normal weight [25-27]. We cannot evaluate the risk of MHAO on diabetes compared with metabolically healthy normal weight since all the subjects in the present study were central obese and none of them could be classified as metabolically healthy or unhealthy normal weight. And because we had a relatively small sample size, we might find the adjusted OR was much higher than those from other [25-27].

Little evidence is available on differences of prevalence of NAFLD or liver fat content between MHO and MUO. In the present study, we found subjects with MUO, compared to those with MHO, showed significantly higher prevalence of NAFLD (90.3% v.s. 69.1%) and IHTG content (16.3±9.5 v.s. 12.3±9.5%) (both p-values<0.001). Our findings indicated the prevalence of NAFLD and IHTG content are still common and high even for those with MHO. Meanwhile MHO is commonly identified based on the presence of obesity and absence of metabolic syndrome, neither NAFLD nor liver fat content has been considered as an additional criterion when defining and diagnosing MHO. Therefore, our findings implied that screening of NAFLD and intervention to reduce IHTG content for those seemly healthy obese should be strengthened.

Our previous findings showed that NAFLD was significantly associated with increased risk of T2DM prevalence [28]. The present study expanded the positive association of NAFLD to risk of prediabetes plus diabetes for all subjects as well as for those with MHAO, and the adjusted ORs (95%CI) were 3.02 (1.47-6.20) and 2.65 (1.25-5.60) (both p-values<0.05),

respectively. NAFLD has been generally diagnosed by hepatic ultrasonography scanning. In the present study, we conducted IHTG content measurement by using magnetic resonance spectroscopy to quantify the extent of liver fat in these abdominal obese subjects. And we found that IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, p=0.024). Moreover, we found a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes. Quantitative MRI proton-density fat fraction method has been proved to serve as accurate noninvasive biomarkers for quantifying liver steatosis [29] and liver fat content was found to be correlated with insulin resistance [30], but evidence was scarce on association between the quantity of liver fat and risk of diabetes. Our results on the association between IHTG content and risks of prediabetes plus diabetes might account for possibly a novel finding for the present study.

We further conducted stratified analyses on the associations of IHTG content with risk of prediabetes plus diabetes for subjects with MHAO and MUAO separately. For those with MHAO, the association of IHTG content with risk of prediabetes plus diabetes was marginally significant, and the adjusted OR (95%CI) of per SD increase of IHTG content was 1.55 (1.00-2.40, p=0.051). With the first tertile of IHTG content as the reference group, the adjusted ORs (95%CI) of risks of prediabetes plus diabetes for the 2nd and 3rd tertiles were 2.31 (1.03-5.17) and 2.81 (1.14-6.90) (both p-values<0.05), respectively. The positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus

diabetes was also statistically significant for the subgroup with MHAO (trend test p-value<0.05). Our findings implied that increased intrahepatic triglyceride content was associated with excessive risk of prediabetes and diabetes even for MHO subjects. To the best of our knowledge, we were probably the first to find the positive associations of IHTG content with risks of diabetes and prediabetes for MHAO subjects. The reason for non-significant results for MUAO subgroups may be due to the relatively small sample size of subjects with MUAO (n=134).

NAFLD and liver fat quantity has not been currently considered in the definitions and diagnose criteria of MHO [24], although liver is one of the main parts of fat accumulation when obesity occurs. The present study found that around 69% of subjects with MHAO were diagnosed as NAFLD. Most importantly, even for these apparently metabolically healthy obese individuals, NAFLD and higher IHTG content were both significantly associated with increased risks of prediabetes plus diabetes. Therefore, our findings implied that the current criteria of MHO may not be appropriate. NAFLD, quantity of liver fat or abdominal fat content (obtained from ultrasonography or CT-scanning techniques) should be considered as additional criterion when defining and diagnosing MHO if more evidence could be proved in future, especially from the prospective cohort studies with larger sample sizes.

A few limitations of the present study should be recognized when generalizing our findings to other populations. Firstly, all subjects were abdominally obese and were not randomly sampled from their living communities; therefore, we could not assess the effect of MHAO as

compared with metabolically healthy non-obesity and we might also under-estimate the true associations of MHAO as compared with MUAO on risks of prediabetes plus diabetes. Secondly, the present analyses were based on the baseline information of our ongoing cohort study, therefore we cannot determine the temporal sequence among MHAO and prediabetes plus diabetes. Thirdly, our sample size was small, especially for the MUAO subgroup and we may not have enough power to determine their true associations. On the other hand, we still have some strengths in the present study. For example, we used IHTG content by magnetic resonance spectroscopy, which was relatively measurement of liver fat. And we were probably the first to find the positive associations of IHTG content with risks of diabetes and prediabetes, especially for subjects with MHAO.

CONCLUSIONS

NAFLD were diagnosed in 69% of MHAO and 90% of MUAO subjects, and the prevalence rates of prediabetes plus diabetes were linearly increased across the tertiles of IHTG content. NAFLD and higher IHTG content were independently associated with increased risks of prediabetes plus diabetes for all subjects as well as for the MHAO subgroups. Therefore, our findings imply that NAFLD or quantity of liver fat should be considered as additional criterion when defining and diagnosing MHO. Furthermore, screening of NAFLD and intervention to reduce liver fat should be strengthened even for the seemly healthy obese subjects.

Declarations

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Author contributions

Q.X., J.Z., F.L., N.C. and Z.L. performed the statistical analysis and wrote the manuscript; M.L. and Y.L. participated in the data collection; S.W.and W.Z. contributed to discussion; C.L., N.C., S.W. and J.Z. participated in the design of the study and edited the manuscript. C.L. and Z.L. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest statement

No potential conflict of interest relevant to this article is declared.

Ethical approval

This study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Xiamen University (Xiamen, China) (number/ID of the obtained ethics approval:

2011YLS-013) and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figure Legends

Figure 1. Study subjects' selection diagram.

Figure 2A. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MHAO subjects.

Figure 2B. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MUAO subjects.

Table Legends

Table 1. Demographic, lifestyle and clinical characteristics of 428 subjects stratified by MHAO and tertiles of IHTG content.

Table 2. Adjusted odds ratios (ORs) with associated 95% confidence interval (CI) of MUAO, NAFLD and IHTG content for prediabetes plus diabetes.

Table 1. Demographic, lifestyle and clinical characteristics of 428 subjects stratified by MHAO and tertiles of IHTG content.

Variables	MHAO (n=294, 68.7%)				MUAO (n=134, 31.3%)				P-value §
	Tertile 1	Tertile 2	Tertile 3	P-value	Tertile 1	Tertile 2	Tertile 3	P-value	
Demographics & life style									
N (%)	98 (33.3%)	98 (33.3%)	98 (33.3%)		45 (33.6%)	45 (33.6%)	44 (32.8%)		
Male gender (n, %)	12 (12.4%)	27 (27.6%)	30 (30.6%)	0.005*	11 (24.4%)	17 (38.8%)	12 (27.3%)	0.347	0.160
Age (years)	52.8±6.5	53.6±6.6	52.4±6.8	0.422	54.8±6.1	54.1±7.5	55.0±6.0	0.821	0.014*
Low educational attainment, (n, %)	61 (62.2%)	48 (49.0%)	51 (52.0%)	0.241	30 (66.7%)	25 (55.6%)	27 (61.4%)	0.110	0.090
Ever smoking (n, %)	16 (16.3%)	25 (25.5%)	23 (23.5%)	0.262	9 (20.0%)	11 (24.4%)	11 (25.0%)	0.828	0.753
Ever drinking (n, %)	8 (8.2%)	13 (13.3%)	15 (15.3%)	0.291	2 (4.4%)	6 (13.3%)	4 (9.1%)	0.216	0.317
Regular physical exercise (n, %)	34 (34.7%)	42 (42.9%)	32 (32.7%)	0.292	13 (28.9%)	17 (37.8%)	10 (22.7%)	0.296	0.165
Clinical characteristics									
IHTG content (%)	4.10±1.63	9.64±1.97	23.25±8.47	<0.001†	6.86±2.44	14.54±2.91	27.68±6.21	<0.001†	<0.001†
NAFLD (n, %)	34 (34.7%)	75 (76.5%)	94 (95.9%)	<0.001†	35 (77.8%)	42 (93.3%)	44 (100.0%)	<0.001†	<0.001†
BMI (kg/m^2)	26.2±2.5	27.2±2.5	28.3±2.7	<0.001†	27.3±2.5	27.7±3.5	28.1±2.8	0.485	0.121
Waist circumference (cm)	90.7±5.1	93.5±6.1	96.0±7.1	<0.001†	94.0±5.3	93.7±7.8	95.3±6.8	0.518	0.158
Systolic blood pressure (mmHg)	125.6±17.3	130.6±17.5	129.5±15.7	0.095	139.8±12.4	140.9±15.3	143.4±12.6	0.451	<0.001†
Diastolic blood pressure (mmHg)	74.0±10.0	77.8±9.8	78.4±9.7	0.004*	82.7±10.7	83.7±9.4	85.2±8.3	0.482	<0.001†
Triglyceride (mmol/L)	1.22±0.57	1.56±0.82	1.89±0.98	<0.001†	2.53±1.26	2.32±1.90	2.84±1.52	0.062	<0.001†
Total cholesterol (mmol/L)	5.80±1.03	5.81±0.95	5.95±1.05	0.487	6.18±1.43	6.13±1.27	6.02±0.94	0.823	0.024*
HDL-cholesterol (mmol/L)	1.50±0.26	1.39±0.29	1.37±0.25	0.003*	1.20±0.19	1.14±0.22	1.18±0.20	0.401	<0.001†
LDL-cholesterol (mmol/L)	3.75±0.95	3.72±0.79	3.75±1.01	0.973	3.83±1.30	3.50±1.41	3.56±0.84	0.391	0.297

Blood uric acid (μmol/L)	322.9±71.5	359.8±83.6	378.2±98.1	<0.001†	348.3±84.2	391.6±90.9	426.0±108.7	<0.001†	0.001*
Fasting plasma glucose (mmol/L)	5.56±0.46	5.55±0.54	5.54±0.51	0.967	5.85±0.44	5.98±0.45	5.94±0.42	0.338	<0.001†
2-h PG (OGTT, mmol/L)	7.42±2.52	7.51±1.86	7.87±1.88	0.285	8.04±1.91	8.09±1.72	8.87±2.13	0.082	<0.001†
HbA1c (%)	5.86±0.29	5.93±0.36	5.97±0.29	0.041*	6.01±0.32	6.00±0.38	6.04±0.36	0.882	0.005*
Diabetes (n, %)	7 (7.1%)	10 (10.2%)	10 (10.2%)	0.693	4 (8.9%)	4 (8.9%)	11 (25.0%)	0.043*	0.122
Prediabetes (n, %)	66 (67.3%)	73 (74.5%)	76 (77.6%)	0.255	39 (86.7%)	40 (88.9%)	32 (72.7%)	0.091	0.039*
Prediabetes plus diabetes (n, %)	73 (74.5%)	83 (84.7%)	86 (87.8%)	0.039*	43 (95.6%)	44 (97.8%)	43 (97.7%)	0.779	<0.001†

* p<0.05, †p<0.001, § P-value for difference between MHAO and MUAO.

All percentages are column percentage; except for percentages, all values are mean±s.d. .

Abbreviations: 2-h PG, 2-hour plasma glucose; BMI, body mass index; HDL, high-density lipoprotein; IHTG, intrahepatic triglyceride; LDL, low-density lipoprotein cholesterol; MHAO, metabolically healthy abdominal obesity; MUAO, metabolically unhealthy abdominal obesity; NAFLD,non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test.

Table 2. Adjusted odds ratios (ORs) with associated 95% confidence interval (CI) of MUAO, NAFLD and IHTG content for prediabetes plus diabetes.

	Prediabetes plus Diabetes		
Variables	OR	95% CI	P-value
All subjects			
MUAO v.s. MHAO	10.90	3.15 - 37.69	<0.001*
NAFLD v.s. Non-NAFLD	3.02	1.47 - 6.20	0.003*
IHTG content (%) †	1.62	1.07 - 2.46	0.024*
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	1.81	0.86 - 3.81	0.117
Tertile 3	3.13	1.28 - 7.61	0.012*
Trend test			0.011*
Interaction test			
MUAO*NAFLD			0.956
MUAO*Tertiles of IHTG			0.869
MHAO subjects			
NAFLD v.s. Non-NAFLD	2.65	1.25 - 5.60	0.011*
IHTG content (%) †	1.55	1.00 - 2.40	0.051
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	2.31	1.03 - 5.17	0.042*
Tertile 3	2.81	1.14 - 6.90	0.024*
Trend test			0.021*
MUAO subjects			
NAFLD v.s. Non-NAFLD	4.77	0.07 - 327.48	0.469
IHTG content (%) †	0.81	0.13 - 5.26	0.830
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	3.22	0.24 - 43.54	0.378
Tertile 3	1.90	0.15 - 23.69	0.620
Trend test			0.558

*p<0.05

† OR and 95%CI was expressed by per SD increase of IHTG content.

‡ OR and 95%CI was expressed by the first quartile of IHTG content as the reference.

Abbreviations: CI, confidence interval; IHTG, intrahepatic triglyceride; MHAO, metabolically healthy abdominal obesity; MUAO, metabolically unhealthy abdominal obesity; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio;

OR was adjusted for age, sex, educational level, ever smoking, ever drinking, physical

activity, BMI, systolic and diastolic BP, triglyceride, total cholesterol, HDL- and LDL-cholesterol and serum uric acid.

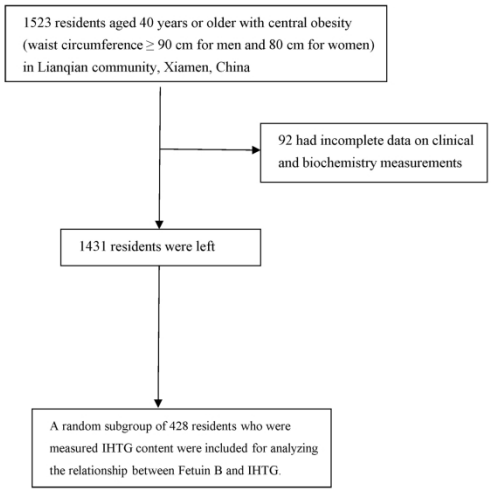

Figure 1. Study subjects' selection diagram.

Figure 1. Study subjects' selection diagram.

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Figure 2A. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MHAO subjects.

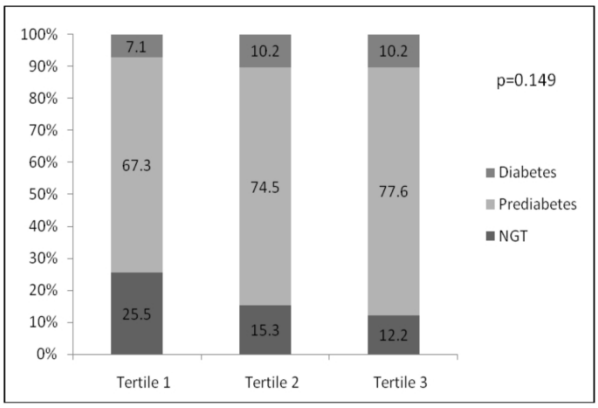

Figure 2B. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MUAO subjects.

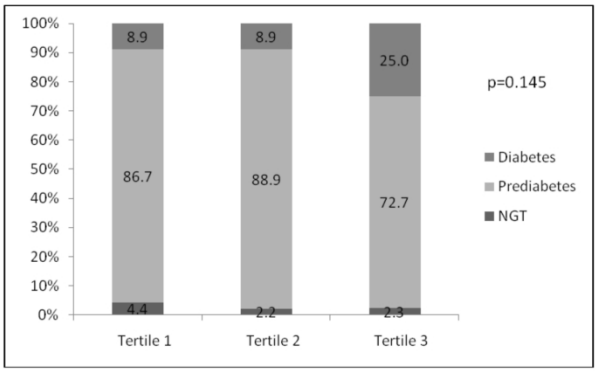

Figure 2A. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MHAO subjects.
Figure 2B. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MUAO subjects.

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

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
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	*7-9*
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
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	12
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	12-13
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	13-14

		(*b*) Report category boundaries when continuous variables were categorized	13-14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-18
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	19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19
Generalisability	21	Discuss the generalisability (external validity) of the study results	19
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	3

*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 between intrahepatic triglyceride content in subjects with metabolically healthy abdominal obesity and risks of prediabetes plus diabetes: an observational study.

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Title Page

Manuscript Type: Original Article

Association between intrahepatic triglyceride content in subjects with metabolically healthy abdominal obesity and risks of prediabetes plus diabetes: an observational study

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ABSTRACT

Objective We aimed to evaluate the association of intrahepatic triglyceride (IHTG) content in subjects with metabolically healthy abdominal obesity (MHAO) on risks of prediabetes plus diabetes.

Design Cross-sectional survey.

Setting Lianqian community, the First Affiliated Hospital of Xiamen University, Xiamen, China.

Participants Among 1,523 community-living healthy adults aged 40 years or older with abdominal obesity recruited at baseline, 428 subjects who underwent intrahepatic triglyceride (IHTG) content measurement were selected.

Outcome measures Risk of prediabetes plus diabetes.

Results Nonalcoholic fatty liver disease (NAFLD) was diagnosed as 203 (69.1%) in MHAO and 121 (90.3%) in metabolically unhealthy abdominal obesity (MUAO) (p<0.001). The prevalence rates of prediabetes plus diabetes were 81.1%, 88.8% and 90.9% across the tertiles of IHTG content (p=0.037). Both MUAO (v.s. MHAO) and NAFLD (v.s. Non-NAFLD) were independently associated with increased risks of prediabetes plus diabetes, the adjusted ORs (95%CIs) were 10.90 (3.15-37.69, p<0.001) and 3.02 (1.47-6.20, p=0.003), respectively. Higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, p=0.024). And there was a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes (trend test p-value =0.011). Stratified analyses showed similar results on the associations of NAFLD

and IHTG content with risks of prediabetes plus diabetes for subjects with MHAO but not for those with MUAO.

Conclusions: NAFLD and higher IHTG content were independently associated with increased risks of prediabetes plus diabetes in MHAO subjects. NAFLD or quantity of liver fat should be considered as additional criterion when defining and diagnosing MHO. Screening of NAFLD and intervention to reduce liver fat should be strengthened even for those seemly metabolically healthy obese.

Keywords: metabolically healthy obesity; intrahepatic triglyceride; nonalcoholic fatty liver disease; prediabetes; diabetes;

Strengths and limitations of this study

- This study was a cross-sectional analysis of baseline information on the ongoing cohort study to evaluate the independent association of IHTG content with risk of prediabetes plus diabetes.
- All subjects were abdominally obese and were not randomly sampled from their living communities.
- IHTG content was determined using magnetic resonance spectroscopy which was relatively quantitative measurement of liver fat.
- The sample size was relatively small, especially for the MUAO subgroup, and we might not have enough power to determine their true associations.

INTRODUCTION

The prevalence of diabetes has quadrupled during the past three decades with an estimated prevalence of 9.3% (463 million people) in 2019 and it is expected to rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [1-3]. Obesity has been well documented to be a risk factor for a broad array of chronic non-communicable diseases, including diabetes, hypertension, coronary heart disease, chronic kidney disease and certain sites of cancer [4-6]. A subgroup of obese individuals who are devoid of obesity-related metabolic complications, such as diabetes and atherosclerosis, arise the concept of metabolically healthy obese (MHO) [7-9]. However, there is no unique definition and diagnose criteria for MHO by now. For example, some defined MHO when two or fewer of the 4 criteria of metabolism syndrome [10] for those obese subjects while others defined as none of them [11], which made evidence on the association of MHO with diabetes was limited and controversial [12].

Nonalcoholic fatty liver disease (NAFLD) is well documented to be associated with risk of diabetes [13], however NAFLD has not been considered as additional criterion for MHO although it usually occurs simultaneously when obesity happens. Therefore, little evidence is available on the risk of NAFLD or liver fat with diabetes for those with MHO. In the present study with 428 community-living Chinese adults with abdominal obesity, we mainly aimed to evaluate associations of intrahepatic triglyceride (IHTG) content and NAFLD in subjects with metabolically healthy abdominal obesity (MHAO) on risks of prediabetes plus diabetes.

METHODS

Study design and subjects

Details on study design and subject recruitment have been described previously [14-16]. Briefly, 1,523 community-living healthy adults aged 40 years or older with abdominal obesity (waist circumference greater than 90 cm for men and 80 cm for women) living in Lianqian community, Xiamen, China were recruited at baseline of the cohort study in 2011. Of them, 92 (6%) who had incomplete data on clinical and biochemistry measurements were excluded, and a random sample of 428 subjects who underwent intrahepatic triglyceride (IHTG) content measurement was left for the present analysis (Figure 1). Of the 428 study subjects, 319 (74.5%) were female with the mean age of 53.6±6.5 years old, 109 (25.5%) were male with the mean age of 53.2±7.1 years old, and there was no significant difference in age between male and female subjects (p=0.592). This study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Xiamen University (Xiamen, China) and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant.

Measurements

Details on study measurements have been described previously [15,16]. For each subject, face-to-face interview was conducted to collect socio-demographic status, lifestyle habits, present and previous history of health and medications. Subjects were excluded if they drank regularly with alcohol consumption ≥140 g/week for men or ≥70 g/week for women, had cancer, or received current treatment with systemic corticosteroids, biliary obstructive

diseases, acute or chronic virus hepatitis, drug-induced liver diseases, total parenteral nutrition, autoimmune hepatitis, Wilson's disease, known hyperthyroidism or hypothyroidism. Subjects underwent weight, height and waist circumference measurements by using a calibrated scale after removing shoes and heavy clothes. Waist circumference was measured at the midpoint between the inferior costal margin and the superior border of the iliac crest on the midaxillary line. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Arterial blood pressure (BP) was measured with a mercury sphygmomanometer after sitting for at least 15 minutes.

Blood samples were obtained after 12-hour fasting and 75-g oral glucose tolerance test were conducted for each subject. All biochemical measurements were tested in the central laboratory of the First Affiliated Hospital, Xiamen University. Plasma glucose and serum lipid profiles, including triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined on a HITACHI 7450 analyzer (HITACHI, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula. Fasting plasma glucose (FPG) and 2-hour plasma glucose (2-h PG) concentrations were measured by the hexokinase method and HbA1c by the Bio-Rad Variant Hemoglobin A1c assay.

Ultrasonography and definition of non-alcoholic fatty liver disease

Details on ultrasonography and definition of NAFLD have been described previously [15,16]. Hepatic ultrasonography scanning was performed by an experienced radiologist using GE

LOGIQ P5 scanner (GE Healthcare, Milwaukee, USA) with a 4-MHz probe, who was blinded to the subjects' health status. Hepatic steatosis was diagnosed on the basis of characteristic sonographic features, including hepatorenal echo contrast, liver parenchymal brightness, deep beam attenuation, and vessel blurring [17]. The definition of NAFLD was based on hepatic ultrasonography diagnosis of hepatic steatosis without excessive alcohol consumption, viral or autoimmune liver disease [15,16].

Intrahepatic triglyceride (IHTG) content measurement

Details on intrahepatic triglyceride (IHTG) content measurement has been described previously [18]. IHTG content was determined by an experienced radiologist using Magnetic resonance spectroscopy (^{1}H MRS, 3.0-T Avanto, Siemens AG, Erlangen, German). Images of a sagittal, coronal and axial cube of a 2 cm^3 volume in the right lobe of liver was acquired. Quantification of the spectra (water and methylene resonances) was performed as described previously [19]. Areas of resonance from water protons and methylene groups in fatty acid chains were obtained with a time-domain non-linear fitting routine by using Syngo MR B15V software (Siemens AG). The percentage of IHTG content was calculated as the ratio of the area under the resonance of peak for methylene groups in fatty acid chains of IHTG and the combined area under the resonance peaks for both methylene groups and water [18,19].

Definition of metabolically healthy abdominal obesity

Abdominal obesity was defined as WC ≥90cm for men and 80cm for women [20]. All subjects in the present study were abdominal obesity which was considered as one of the

recruitment criteria. Subjects were defined as metabolically healthy abdominal obesity (MHAO) if two or fewer of the following criteria were met: ①systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg; ②fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L); ③TG ≥ 150 mg/dL (1.7 mmol/L); ④HDL cholesterol < 40 mg/dL (1.03 mmol/L) in men and < 50 mg/dL (1.30 mmol/L) in women [16,21,22]. Otherwise, subjects meeting 3 or more of the criteria were defined as metabolically unhealthy abdominal obesity (MUAO). Therefore, all subjects in the present study were dichotomized as either MHAO or MUAO.

Definitions of diabetes and prediabetes

According to American Diabetes Association (ADA) 2020 criteria, diabetes was defined as (1) a self-reported history of diabetes previously diagnosed by health care professionals; (2) FGP ≥126 mg/dL (7.0mmol/L); (3) 2-hour plasma glucose (2-h PG, OGTT) ≥200 mg/dL (11.1mmol/L); or (4) HbA1c ≥6.5%. Prediabetes was defined as (1) FPG levels between 100 mg/dL (5.6mmol/L) and 125 mg/ dL (6.9mmol/L), (2) 2-h PG levels between 140 mg/dL (7.8mmol/L) and 199 mg/dL (11.0mmol/L), or (3) HbA1c between 5.7% and 6.4% in participants without a prior diabetes diagnosis [15,16,23].

Statistical analyses

Methods on statistical analyses were similar to our previous publications [15,16,18]. Data were presented as the mean ± standard deviation for continuous variables or number and percentage for categorical variables. Skewness and kurtosis tests for continuous variables were conducted and found them followed approximation of normal distributions. Differences

between subjects categorized by MHAO and tertiles of IHTG content were analyzed using one-way ANOVA for continuous variables and chi-square test for categorical variables. Bar graphs showing prevalence rates of diabetes, prediabetes and normal glucose test (NGT) were made by MHAO (v.s. MUAO) and tertiles of IHTG content.

Multivariable logistic regression models were used to calculate the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of abdominal obesity (MUAO v.s. MHAO), NAFLD (yes v.s. no) and IHTG content (both the originally continuous values and the tertiles categories) for prediabetes plus diabetes with adjustment for potential confounders (including age, sex, educational level, smoking and drinking habits, regular physical exercise, BMI, systolic and diastolic BP, triglyceride, total cholesterol, HDL- and LDL-cholesterol and serum uric acid). And multivariable logistic regression analyses stratified by MHAO and MUAO groups were further conducted. All p-values were two-sided and p-value <0.05 was considered statistically significant. All statistical analyses were performed using Stata14.0 (StatCorp, College Station, TX).

Patient and public involvement

There were no funds or time allocated for patient and public involvement.

RESULTS

Prevalence of diabetes and prediabetes stratified by MHAO and tertiles of IHTG content

Among the 428 subjects with abdominal obesity, MHAO and MUAO were identified on 294 (68.7%) and 134 (31.3%) subjects. Of them, 46 (10.8%), 326 (76.2%) and 56 (13.1%) were diagnosed as diabetes, prediabetes and normal glucose tolerance (NGT), respectively. There was a significantly positive trend between increasing tertiles of IHTG content and higher prevalence of prediabetes plus diabetes (81.1%, 88.8% and 90.9% across the tertiles of IHTG content (p=0.037)). Figure 2A showed the prevalence rates of diabetes and prediabetes across the tertiles of IHTG content in MHAO subjects were 7.1% and 67.3%, 10.2% and 74.5%, 10.2% and 77.6% for the Tertile 1, Tertile 2 and Tertile 3, respectively (p-value>0.05). But there was a significantly positive trend of higher prevalence of diabetes plus diabetes with increasing categories of tertiles of IHTG content (p=0.039). Figure 2B showed the prevalence rates of diabetes and prediabetes across the tertiles of IHTG content in MUAO subjects were 8.9% and 86.7%, 8.9% and 88.9%, 25.0% and 72.7% for Tertile 1, Tertile 2 and Tertile 3, respectively. Table 1 also showed MUAO subjects had significantly higher prevalence of prediabetes and prediabetes plus diabetes than MHAO subjects (both p-values<0.05).

Demographic and clinical characteristics stratified by MHAO and tertiles of IHTG content

For all the 428 subjects, the means (±SD) of age were 53.6 (±6.5) years for women (n=319, 74.5%) and 53.2 (±7.1) years for men (n=109, 25.5%) (p=0.592). Table 1 showed differences

of demographics, life style habits and clinical characteristics stratified by MHAO and tertiles of IHTG content. For 294 MHAO subjects, with increasing categories of the tertiles of IHTG content (from tertile 1, tertile 2 to tertile 3), subjects were more likely to be male and had significantly higher levels of indices of obesity (BMI, waist circumference), diastolic BP, triglyceride, HbA1c, serum uric acid as well as higher prevalence of NAFLD and significantly lower level of HDL-C. As for 134 MUAO subjects, increasing categories of the tertiles of IHTG content were significantly related to higher prevalence of NAFLD and serum uric acid levels. Furthermore, Table 1 showed that, compared to subjects with MHAO, those with MUAO had significantly increased age, IHTG content, prevalence of NAFLD, systolic and diastolic BP, triglyceride, total cholesterol, FPG, 2-h PG, HbA1c, serum uric acid and significantly lower level of HDL-C.

Associations of MHAO, NAFLD and IHTG content with prediabetes plus diabetes for all subjects

Table 2 showed that, for all subjects, both MUAO (v.s. MHAO) and NAFLD (yes v.s. no) were independently associated with increased risk of prediabetes plus diabetes, and the adjusted ORs (95%CIs) were 10.90 (3.15-37.69, p<0.001) and 3.02 (1.47-6.20, p=0.003), respectively. Higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, p=0.024). With the tertile 1 of IHTG content as the reference, the tertile 3 showed significantly higher risk of prediabetes plus diabetes (adjusted OR (95%CI): 3.13 (1.28-7.61), p=0.012). And there was a significantly positive trend of increasing categories of

IHTG content tertiles with excessive risk of prediabetes plus diabetes (trend test: p=0.011). There was no significant interaction between MHAO with either NAFLD or tertiles of IHTG content for risk of prediabetes plus diabetes (both p-values>0.05).

Stratified analyses on associations of NAFLD and IHTG content with prediabetes plus diabetes by MHAO & MUAO

Multivariable logistic regression analyses stratified by MHAO and MUAO separately were conducted (Table 2). For MHAO subjects, NAFLD was independently associated with increased risk of prediabetes plus diabetes (adjusted OR (95%CI): 2.65 (1.25-5.60), p=0.011). Per SD increase of IHTG content was marginally associated with excessive risk of prediabetes plus diabetes with the adjusted OR (95%CI) of 1.55 (1.00-2.40, p=0.051). Compared with the tertile 1 of IHTG content, both the tertile 2 and tertile 3 groups showed significantly increased risks of prediabetes plus diabetes with the adjusted ORs (95%CI) of 2.31 (1.03-5.17, p=0.042) and 2.81 (1.14-6.90, p=0.024), respectively. And there was also a significantly positive trend between increasing categories of IHTG content tertiles and excessive risk of prediabetes plus diabetes (trend test: p=0.021). For MUAO subjects, neither NAFLD nor IHTG content was found to be significantly associated with risk of prediabetes plus diabetes.

DISCUSSION

In the present study of 428 subjects with abdominal obesity, 294 (68.7%) and 134 (31.3%) were identified as MHAO and MUAO, respectively. Both MUAO (v.s. MHAO) and NAFLD (v.s. Non-NAFLD) were independently associated with increased risks of prediabetes plus diabetes. Furthermore, higher IHTG content was significantly associated with increased risk of prediabetes plus diabetes, and there was a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes. Stratified analyses showed similar results for subjects with MHAO but not for those with MUAO.

The concept of MHO has been established for a subgroup of obese subjects who do not exhibit metabolic and cardiovascular complications at a given time point, such as diabetes and atherosclerosis, for a few decades [24,25]. Compared to subjects with MUO, those with MHO are characterized by lower liver and visceral fat, higher subcutaneous leg fat, greater cardiorespiratory fitness, physical activity and insulin sensitivity, lower levels of inflammation, and normal adipose tissue function [26]. However, it could be debated whether MHO predicts the risk of diabetes compared with metabolically healthy normal weight or MUO. Hinnouho GM et al, based on the Whitehall II cohort study, found a significantly decreased risk of diabetes for MHO compared with metabolically unhealthy obesity (MUO) (HR=1.98 (MUO v.s. MHO), 95% CI: 1.39–2.83) [27]. The present study found similar results that MUAO was significantly associated with increased risk of prediabetes plus diabetes compared with MHAO but with a much higher adjusted OR(95%CI) (10.90 (3.15-37.69)). Hinnouho GM et al and others further found that MHO showed a significant

increased risk of T2DM incidence compared with metabolically healthy normal weight [27-29]. We cannot evaluate the risk of MHAO on diabetes compared with metabolically healthy normal weight since all the subjects in the present study were central obese and none of them could be classified as metabolically healthy or unhealthy normal weight. And because we had a relatively small sample size, we might find the adjusted OR was much higher than those from other [27-29].

Little evidence is available on differences of prevalence of NAFLD or liver fat content between MHO and MUO. In the present study, we found subjects with MUO, compared to those with MHO, showed significantly higher prevalence of NAFLD (90.3% v.s. 69.1%) and IHTG content (16.3±9.5 v.s. 12.3±9.5%) (both p-values<0.001). Our findings indicated the prevalence of NAFLD and IHTG content are still common and high even for those with MHO. Meanwhile MHO is commonly identified based on the presence of obesity and absence of metabolic syndrome, neither NAFLD nor liver fat content has been considered as an additional criterion when defining and diagnosing MHO. Therefore, our findings implied that screening of NAFLD and intervention to reduce IHTG content for those seemly healthy obese should be strengthened.

Our previous findings showed that NAFLD was significantly associated with increased risk of T2DM prevalence [15]. The present study expanded the positive association of NAFLD to risk of prediabetes plus diabetes for all subjects as well as for those with MHAO, and the adjusted ORs (95%CI) were 3.02 (1.47-6.20) and 2.65 (1.25-5.60) (both p-values<0.05),

respectively. NAFLD has been generally diagnosed by hepatic ultrasonography scanning. In the present study, we conducted IHTG content measurement by using magnetic resonance spectroscopy to quantify the extent of liver fat in these abdominal obese subjects. And we found that IHTG content was significantly associated with increased risk of prediabetes plus diabetes with the adjusted OR (95%CI) of per SD increase of IHTG content of 1.62 (1.07-2.46, p=0.024). Moreover, we found a significantly positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus diabetes. Quantitative MRI proton-density fat fraction method has been proved to serve as accurate noninvasive biomarkers for quantifying liver steatosis [30] and liver fat content was found to be correlated with insulin resistance [31], but evidence was scarce on association between the quantity of liver fat and risk of diabetes. Our results on the association between IHTG content and risks of prediabetes plus diabetes might account for possibly a novel finding for the present study.

We further conducted stratified analyses on the associations of IHTG content with risk of prediabetes plus diabetes for subjects with MHAO and MUAO separately. For those with MHAO, the association of IHTG content with risk of prediabetes plus diabetes was marginally significant, and the adjusted OR (95%CI) of per SD increase of IHTG content was 1.55 (1.00-2.40, p=0.051). With the first tertile of IHTG content as the reference group, the adjusted ORs (95%CI) of risks of prediabetes plus diabetes for the 2nd and 3rd tertiles were 2.31 (1.03-5.17) and 2.81 (1.14-6.90) (both p-values<0.05), respectively. The positive trend between increasing categories of IHTG content tertiles and excessive risks of prediabetes plus

diabetes was also statistically significant for the subgroup with MHAO (trend test p-value<0.05). Our findings implied that increased intrahepatic triglyceride content was associated with excessive risk of prediabetes and diabetes even for MHO subjects. To the best of our knowledge, we were probably the first to find the positive associations of IHTG content with risks of diabetes and prediabetes for MHAO subjects. The reason for non-significant results for MUAO subgroups may be due to the relatively small sample size of subjects with MUAO (n=134).

NAFLD and liver fat quantity has not been currently considered in the definitions and diagnose criteria of MHO [16,26], although liver is one of the main parts of fat accumulation when obesity occurs. The present study found that around 69% of subjects with MHAO were diagnosed as NAFLD. Most importantly, even for these apparently metabolically healthy obese individuals, NAFLD and higher IHTG content were both significantly associated with increased risks of prediabetes plus diabetes. Therefore, our findings implied that the current criteria of MHO may not be appropriate. NAFLD, quantity of liver fat or abdominal fat content (obtained from ultrasonography or CT-scanning techniques) should be considered as additional criterion when defining and diagnosing MHO if more evidence could be proved in future, especially from the prospective cohort studies with larger sample sizes.

A few limitations of the present study should be recognized when generalizing our findings to other populations. Firstly, all subjects were abdominally obese and were not randomly sampled from their living communities; therefore, we could not assess the effect of MHAO as

compared with metabolically healthy non-obesity and we might also under-estimate the true associations of MHAO as compared with MUAO on risks of prediabetes plus diabetes. Secondly, the present analyses were based on the baseline information of our ongoing cohort study, therefore we cannot determine the temporal sequence among MHAO and prediabetes plus diabetes. Thirdly, our sample size was small, especially for the MUAO subgroup and we may not have enough power to determine their true associations. On the other hand, we still have some strengths in the present study. For example, we used IHTG content by magnetic resonance spectroscopy, which was relatively measurement of liver fat. And we were probably the first to find the positive associations of IHTG content with risks of diabetes and prediabetes, especially for subjects with MHAO.

CONCLUSIONS

NAFLD were diagnosed in 69% of MHAO and 90% of MUAO subjects, and the prevalence rates of prediabetes plus diabetes were linearly increased across the tertiles of IHTG content. NAFLD and higher IHTG content were independently associated with increased risks of prediabetes plus diabetes for all subjects as well as for the MHAO subgroups. Therefore, our findings imply that NAFLD or quantity of liver fat should be considered as additional criterion when defining and diagnosing MHO. Furthermore, screening of NAFLD and intervention to reduce liver fat should be strengthened even for the seemly healthy obese subjects.

Declarations

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Author contributions

Q.X., J.Z., H.H., N.C. and Z.L. performed the statistical analysis and wrote the manuscript; H.H., F.L., Y.L., M.L. and C.H. participated in the data collection; S.W. and W.Z. contributed to discussion; C.L., N.C., S.W. and J.Z. participated in the design of the study and edited the manuscript. C.L. and Z.L. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest statement

No potential conflict of interest relevant to this article is declared.

Ethical approval

This study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Xiamen University (Xiamen, China) (number/ID of the obtained ethics approval:

2011YLS-013) and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figure Legends

Figure 1. Study subjects' selection diagram.

Figure 2A. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MHAO subjects.

Figure 2B. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MUAO subjects.

Table Legends

Table 1. Demographic, lifestyle and clinical characteristics of 428 subjects stratified by MHAO and tertiles of IHTG content.

Table 2. Adjusted odds ratios (ORs) with associated 95% confidence interval (CI) of MUAO, NAFLD and IHTG content for prediabetes plus diabetes.

Table 1. Demographic, lifestyle and clinical characteristics of 428 subjects stratified by MHAO and tertiles of IHTG content.

Variables	MHAO (n=294, 68.7%)				MUAO (n=134, 31.3%)				P-value §
	Tertile 1	Tertile 2	Tertile 3	P-value	Tertile 1	Tertile 2	Tertile 3	P-value	
Demographics & life style									
N (%)	98 (33.3%)	98 (33.3%)	98 (33.3%)		45 (33.6%)	45 (33.6%)	44 (32.8%)		
Male gender (n, %)	12 (12.4%)	27 (27.6%)	30 (30.6%)	0.005*	11 (24.4%)	17 (38.8%)	12 (27.3%)	0.347	0.160
Age (years)	52.8±6.5	53.6±6.6	52.4±6.8	0.422	54.8±6.1	54.1±7.5	55.0±6.0	0.821	0.014*
Low educational attainment, (n, %)	61 (62.2%)	48 (49.0%)	51 (52.0%)	0.241	30 (66.7%)	25 (55.6%)	27 (61.4%)	0.110	0.090
Ever smoking (n, %)	16 (16.3%)	25 (25.5%)	23 (23.5%)	0.262	9 (20.0%)	11 (24.4%)	11 (25.0%)	0.828	0.753
Ever drinking (n, %)	8 (8.2%)	13 (13.3%)	15 (15.3%)	0.291	2 (4.4%)	6 (13.3%)	4 (9.1%)	0.216	0.317
Regular physical exercise (n, %)	34 (34.7%)	42 (42.9%)	32 (32.7%)	0.292	13 (28.9%)	17 (37.8%)	10 (22.7%)	0.296	0.165
Clinical characteristics									
IHTG content (%)	4.10±1.63	9.64±1.97	23.25±8.47	<0.001†	6.86±2.44	14.54±2.91	27.68±6.21	<0.001†	<0.001†
NAFLD (n, %)	34 (34.7%)	75 (76.5%)	94 (95.9%)	<0.001†	35 (77.8%)	42 (93.3%)	44 (100.0%)	<0.001†	<0.001†
BMI (kg/m^2)	26.2±2.5	27.2±2.5	28.3±2.7	<0.001†	27.3±2.5	27.7±3.5	28.1±2.8	0.485	0.121
Waist circumference (cm)	90.7±5.1	93.5±6.1	96.0±7.1	<0.001†	94.0±5.3	93.7±7.8	95.3±6.8	0.518	0.158
Systolic blood pressure (mmHg)	125.6±17.3	130.6±17.5	129.5±15.7	0.095	139.8±12.4	140.9±15.3	143.4±12.6	0.451	<0.001†
Diastolic blood pressure (mmHg)	74.0±10.0	77.8±9.8	78.4±9.7	0.004*	82.7±10.7	83.7±9.4	85.2±8.3	0.482	<0.001†
Triglyceride (mmol/L)	1.22±0.57	1.56±0.82	1.89±0.98	<0.001†	2.53±1.26	2.32±1.90	2.84±1.52	0.062	<0.001†
Total cholesterol (mmol/L)	5.80±1.03	5.81±0.95	5.95±1.05	0.487	6.18±1.43	6.13±1.27	6.02±0.94	0.823	0.024*
HDL-cholesterol (mmol/L)	1.50±0.26	1.39±0.29	1.37±0.25	0.003*	1.20±0.19	1.14±0.22	1.18±0.20	0.401	<0.001†
LDL-cholesterol (mmol/L)	3.75±0.95	3.72±0.79	3.75±1.01	0.973	3.83±1.30	4.50±1.41	3.56±0.84	0.391	0.297

Blood uric acid (μmol/L)	322.9±71.5	359.8±83.6	378.2±98.1	<0.001†	348.3±84.2	391.6±90.9	426.0±108.7	<0.001†	0.001*
Fasting plasma glucose (mmol/L)	5.56±0.46	5.55±0.54	5.54±0.51	0.967	5.85±0.44	5.98±0.45	5.94±0.42	0.338	<0.001†
2-h PG (OGTT, mmol/L)	7.42±2.52	7.51±1.86	7.87±1.88	0.285	8.04±1.91	8.09±1.72	8.87±2.13	0.082	<0.001†
HbA1c (%)	5.86±0.29	5.93±0.36	5.97±0.29	0.041*	6.01±0.32	6.00±0.38	6.04±0.36	0.882	0.005*
Diabetes (n, %)	7 (7.1%)	10 (10.2%)	10 (10.2%)	0.693	4 (8.9%)	4 (8.9%)	11 (25.0%)	0.043*	0.122
Prediabetes (n, %)	66 (67.3%)	73 (74.5%)	76 (77.6%)	0.255	39 (86.7%)	40 (88.9%)	32 (72.7%)	0.091	0.039*
Prediabetes plus diabetes (n, %)	73 (74.5%)	83 (84.7%)	86 (87.8%)	0.039*	43 (95.6%)	44 (97.8%)	43 (97.7%)	0.779	<0.001†

* p<0.05, †p<0.001, § P-value for difference between MHAO and MUAO.

All percentages are column percentage; except for percentages, all values are mean±s.d. .

Abbreviations: 2-h PG, 2-hour plasma glucose; BMI, body mass index; HDL, high-density lipoprotein; IHTG, intrahepatic triglyceride; LDL, low-density lipoprotein cholesterol; MHAO, metabolically healthy abdominal obesity; MUAO, metabolically unhealthy abdominal obesity; NAFLD,non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test.

Table 2. Adjusted odds ratios (ORs) with associated 95% confidence interval (CI) of MUAO, NAFLD and IHTG content for prediabetes plus diabetes.

	Prediabetes plus Diabetes		
Variables	OR	95% CI	P-value
All subjects			
MUAO v.s. MHAO	10.90	3.15 - 37.69	<0.001*
NAFLD v.s. Non-NAFLD	3.02	1.47 - 6.20	0.003*
IHTG content (%) †	1.62	1.07 - 2.46	0.024*
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	1.81	0.86 - 3.81	0.117
Tertile 3	3.13	1.28 - 7.61	0.012*
Trend test			0.011*
Interaction test			
MUAO*NAFLD			0.956
MUAO*Tertiles of IHTG			0.869
MHAO subjects			
NAFLD v.s. Non-NAFLD	2.65	1.25 - 5.60	0.011*
IHTG content (%) †	1.55	1.00 - 2.40	0.051
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	2.31	1.03 - 5.17	0.042*
Tertile 3	2.81	1.14 - 6.90	0.024*
Trend test			0.021*
MUAO subjects			
NAFLD v.s. Non-NAFLD	4.77	0.07 - 327.48	0.469
IHTG content (%) †	0.81	0.13 - 5.26	0.830
IHTG content tertiles ‡			
Tertile 1	1.00		
Tertile 2	3.22	0.24 - 43.54	0.378
Tertile 3	1.90	0.15 - 23.69	0.620
Trend test			0.558

*p<0.05

† OR and 95%CI was expressed by per SD increase of IHTG content.

‡ OR and 95%CI was expressed by the first quartile of IHTG content as the reference.

Abbreviations: CI, confidence interval; IHTG, intrahepatic triglyceride; MHAO, metabolically healthy abdominal obesity; MUAO, metabolically unhealthy abdominal obesity; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio;

OR was adjusted for age, sex, educational level, ever smoking, ever drinking, physical

activity, BMI, systolic and diastolic BP, triglyceride, total cholesterol, HDL- and LDL-cholesterol and serum uric acid.

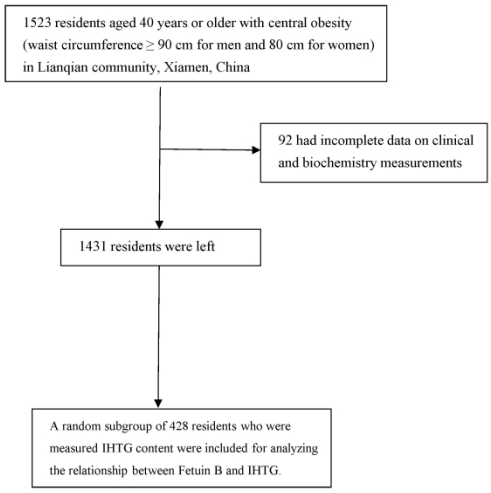

Figure 1. Study subjects' selection diagram.

Figure 1. Study subjects' selection diagram.

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Figure 2A. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MHAO subjects.

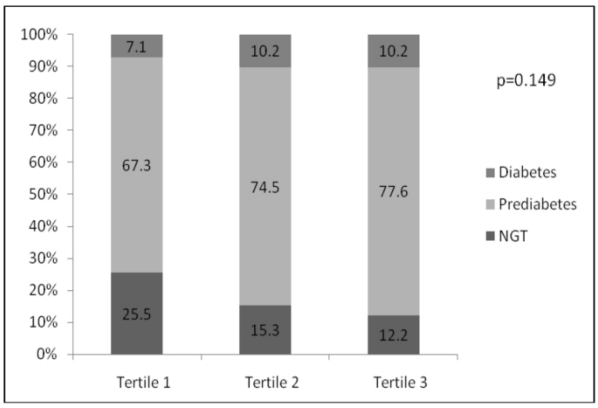

Figure 2B. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MUAO subjects.

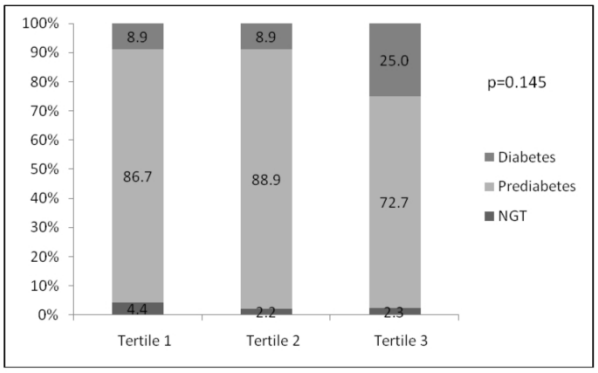

Figure 2A. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MHAO subjects.
Figure 2B. Prevalence rates (%) of prediabetes and diabetes stratified by Tertiles of IHTG content in MUAO subjects.

209x296mm (400 x 400 DPI)

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

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
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	*7-9*
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
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	12
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	12-13
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	13-14

		(*b*) Report category boundaries when continuous variables were categorized	13-14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-18
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	19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19
Generalisability	21	Discuss the generalisability (external validity) of the study results	19
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	3

*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.