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Triglycerides Mediate the Influence of Body Mass Index on Non-Alcoholic Fatty Liver Disease in a Non-Obese Chinese Population with Normal Low-Density Lipoprotein Cholesterol Levels

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Keywords
Non-obese non-alcoholic fatty liver disease · Body mass index · Triglycerides · Insulin resistance · Redox imbalance

Abstract

Introduction: Over 25% of the world's population has non-obese or lean non-alcoholic fatty liver disease (NAFLD), and the prevalence is higher than average in Asia. The present study focused on the relationship between body mass index (BMI) and non-obese NAFLD in non-overweight people in China, particularly the influence of triglycerides (TG) in the pathogenesis of non-obese NAFLD. The findings suggest new treatments for NAFLD patients with normal BMI, as well as provide an early warning system for the understanding and prevention of NAFLD in non-obese patients. ***Methods:*** This cross-sectional study enrolled 159,959 Chinese subjects with BMI <24 kg/m^2 and normal levels of low-density lipoprotein cholesterol (LDL-c). The average age was 40.21 ± 13.88 years, and males accounted for 45.7%. A total of 15,907 (9.94%) patients with NAFLD were diagnosed by ultrasonography. Biochemical indicators were measured using an automated analyzer (Abbott AxSYM). The BMI (kg/m^2) was calculated from the weight (kg)/height in square meters (m^2). The BMI quartile was used as the column-stratified variable to determine the baseline distribution, and logistic regression analysis was used to assess the relationship between NAFLD and its risk factors, with multiple logistic regression used to assess the relationships between BMI or TG and NAFLD and multivariate linear regression used to analyze the association between BMI and TG, while mediation analysis was used to assess the mediation effect of TG. ***Results:*** After adjustment of all covariates, the odds ratios were 1.788 (95% CI: 1.749–1.829; $p < 0.00001$) and 1.491 (95% CI: 1.451–1.532; $p < 0.00001$) for the association between BMI and TG with NAFLD incidence. The multivariate linear regression coefficient of BMI and TG was β = 0.027 (95% CI: 0.023–0.030; $p < 0.00001$). Mediation analysis showed that BMI contributed to 10.81% of lean NAFLD with a mediation effect of 2.98%. ***Conclusion:*** In a Chinese population with BMI <24 kg/m^2 and normal LDL-c levels, BMI and TG were found to be independent predictors of NAFLD. The direct effect of BMI on non-obese NAFLD was 10.41%. The TG level was found to partially mediate the association.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases. It refers to a clinicopathological syndrome characterized by liver steatosis that is not caused by excessive drinking, viruses, or drugs. The syndrome includes non-alcoholic steatohepatitis (NASH) and is often accompanied by inflammation, liver cell injury, cirrhosis, and even hyperplastic carcinoma and death [1, 2]. It is estimated that about one-quarter of the world's population suffers from NAFLD, which is frequently accompanied by type 2 diabetes mellitus, hyperlipidemia, chronic kidney disease, or cardiovascular disease [3]. This represents a major health problem in Western countries, and NAFLD and its complications are also increasing in prevalence in Asia, particularly in China. The situation regarding the prevention and treatment of NAFLD in China is considered serious [4, 5].

According to statistics, the prevalence of non-obese NAFLD has increased every year, and about 10–20% of all NAFLD patients have normal body mass index (BMI); these cases are referred to as "lean" or "non-obese" NAFLD [6]. It is widely recognized that non-obese NAFLD can develop into steatohepatitis and tissue fibrosis [7]. Non-obese NAFLD also has an even higher mortality rate and faster disease progression than NAFLD in obese patients [8], and non-obese NAFLD patients with abnormal liver biochemical parameters may also be prone to severe tissue fibrosis [9]. The prevalence of non-obese NAFLD in Asia is about 30%; although the average BMI of Asians is lower than that of other races, their percentage of visceral fat is higher on average and they are more prone to develop insulin resistance (IR). Slight fluctuations in body weight may lead to metabolic disorders, which can cause non-obese people to develop NAFLD [10, 11]. Oxidative stress may play a central role in the development and progression of NAFLD. In NAFLD, redox imbalance can lead to polyunsaturated fatty acid (PUFA) depletion, which in turn leads to downregulation of the peroxisome proliferator-activated receptor alpha (PPAR-α), decreased fatty acid oxidation, and IR, which can further exacerbate the inflammatory response in NASH and worsen the condition [12]. NAFLD is mainly caused by unhealthy lifestyles such as poor diets, and dietary changes represent the first-line intervention measures. Low carbohydrate and fructose intake, as well as a Mediterranean diet rich in n-3 polyunsaturated fatty acids (n-3 PUFAs), can reduce IR, and isoflavones in soy products can improve the antioxidant capacity and reduce liver fat deposition [13].

An abnormal lipid balance in the liver leads to overaccumulation of triglycerides (TG), and patients with NAFLD often have elevated TG levels [14]. However, in the non-obese population, "marginally elevated" TG levels and even normal levels of TG are also associated with NAFLD. In Chinese people with BMI <25 kg/m^2, both BMI and TG are risk factors for NAFLD [15]. In obese people, TG can mediate the influence of BMI on NAFLD [16], and metabolic dysfunction, including dysfunction associated with obesity and dyslipidemia, is closely associated with the pathogenesis of NAFLD [17]. However, the relationships between BMI, TG, and NAFLD in non-obese people are still controversial and unclear. Therefore, we conducted a cross-sectional study on a non-obese Chinese population, searching the Dryad database to explore whether BMI and TG are correlated with NAFLD in non-overweight Chinese people (BMI <24 kg/m^2) and whether TG mediates the impact of BMI on NAFLD.

Methods

Data Source

The original research data provided by Sun et al. [18] were downloaded free of charge from the DATADRYAD database. We have obtained the consent of Professor Zheng, the author of this article.

Study Population

This cross-sectional study initially recruited 339,101 participants for physical examination in Wenzhou People's Hospital between January 2010 and December 2014. According to the inclusion criteria, 183,903 non-obese participants were included in the analysis. According to the World Health Organization (WHO) classification of BMI, BMI values ≥25 are considered overweight. However, as our research population was Chinese, we used the Chinese index which considers overweight as having BMI ≥24 kg/m^2. So we excluded all overweight patients with BMI ≥24 kg/m^2 and an abnormal value of BUN = 540 mmol/L to finally include 159,959 participants. The Ethics Committee of Wenzhou People's Hospital approved the study, and oral informed consent was obtained from all participants (Fig. 1).

Study Design

In terms of the cross-sectional design of the study, the outcome variable was NAFLD, the exposure variable was BMI, and the intermediary variable was TG. The aim was to analyze the correlation between BMI and NAFLD in a non-overweight Chinese population and evaluate to what extent TG influences this correlation.

Variables

The outcome variable was NAFLD (0 = non-NAFLD, 1 = NAFLD), the exposure variable was BMI, and the intermediary variable was TG. The covariates were the categorical variable of

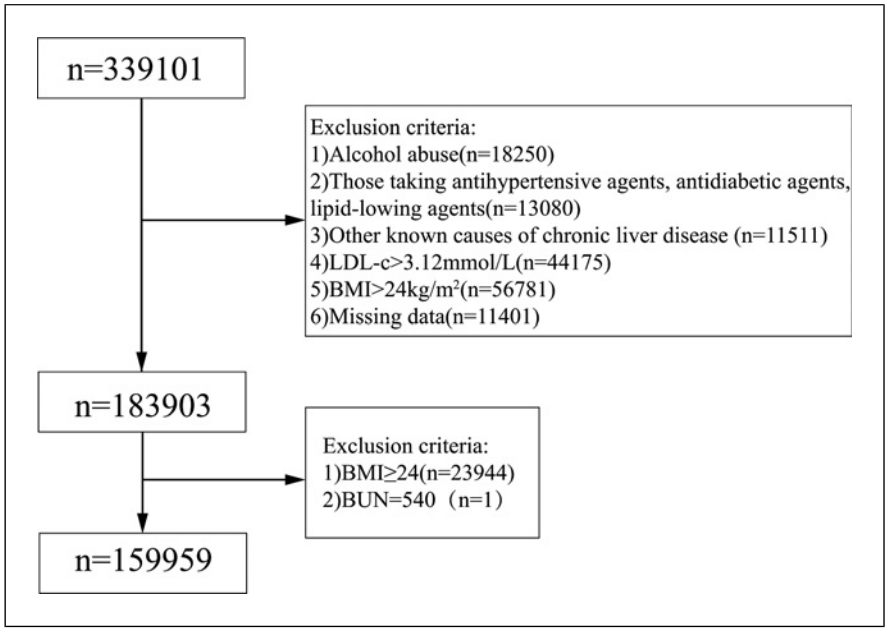

Fig. 1. The flowchart of the enrolled participants.

sex, while continuous variables were age, γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), albumin (ALB), globulin (GLB), total bilirubin (TB), blood urea nitrogen (BUN), creatinine (Scr), uric acid (UA), fasting plasma glucose (FPG), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c).

To diagnose NAFLD using ultrasonography, we referred to the ultrasound criteria of the Chinese Liver Disease Association [19]. NAFLD is defined as diffuse enhancement of the near-field echo and attenuation of the far-field echo in the hepatic region compared with the spleen and kidney regions. It may include any combination of the following items: (1) slight to moderate intrahepatic enlargement with a rounded and blunt boundary; (2) incomplete right hepatic lobe and diaphragm capsule; (3) vague intrahepatic lacuna structure; (4) good blood flow distribution and decreased blood flow signal. All biochemical indicators were measured using an automated analyzer (Abbott AxSYM, Chicago, IL, USA), and the medical history and health habits of the subjects were ascertained by interview.

Statistical Analysis

The quartile of BMI was used as a column-stratified variable to observe the distribution of the baseline data. Normally distributed continuous variables are represented as mean ± standard deviation (SD), while non-normally distributed variables are presented as medians (Q1–Q3), and categorical variables are shown as frequencies (percentage). One-way analysis of variance (ANOVA) was used to analyze normally distributed continuous variables, with Kruskal-Wallis *H* tests used for non-normally distributed continuous variables, and categorical variables were analyzed by χ^2 tests. Logistic regression was used to analyze the correlation between NAFLD and its risk factors without adjustment of any variables, while multiple logistic regression analysis was used to evaluate the relevance between BMI and TG with NAFLD. Model I adjusted for sex and age, and model II adjusted for the covariates sex, age, GGT, AST, ALT, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, LDL-c, and HDL-c. In addition, we conducted sensitivity analysis, dividing the BMI and TG values into four groups according to continuous variables, followed by analysis of the trend. The relationship between BMI and TG was analyzed using multivariate linear regression, and the result is represented by β. Model I adjusted for sex and age, while Model II adjusted for sex, age, NAFLD, GGT, AST, ALT, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, LDL-c, and HDL-c. Causal mediator effect analysis was used to explore whether TG mediated the relationship between BMI and NAFLD, and after adjusting for sex, age, GGT, AST, ALT, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, LDL-c, and HDL-c, the natural direct effect, natural indirect effect (NIE), and the percent mediation (PM) total effect were obtained. The percent mediation was obtained by dividing the NDI by the total effect, indicating the extent to which the TG level mediated the impact of BMI on NAFLD [20, 21]. We used the statistical packages R (The R Foundation; http://www.r-project.org; version 4.2.0) and EmpowerStats (www.empowerstats.net, X&Y Solutions, Boston, MA, USA) for data analysis.

Results

Baseline Characteristics of Participants

A total of 159,959 participants with BMI <24 kg/m^2 were included in the analysis. Table 1 illustrates the baseline characteristics of the participants stratified according to their BMI quartiles: 18.61 (17.867–19.142) kg/m^2, 20.393 (20.01–20.76) kg/m^2, 21.842 (21.49–22.19) kg/m^2, and 23.256 (22.91–23.63) kg/m^2. The corresponding ages of the participants were 32.000 (26.00–39.00) years,

Table 1. Baseline characteristics of participants (*N* = 159,959)

Characteristics	BMI quartile, kg/m^2				*p* value
	Q1 18.61 (17.867–19.142)	Q2 20.393 (20.01–20.76)	Q3 21.842 (21.49–22.19)	Q4 23.256 (22.91–23.63)	
N	39,956	39,997	40,009	39,997	
Sex, *n* (%)					<0.001
Female	28,985 (72.542)	25,255 (63.142)	19,375 (48.427)	13,246 (33.117)	
Male	10,971 (27.458)	14,742 (36.858)	20,634 (51.573)	26,751 (66.883)	
NAFLD, *n* (%)					<0.001
No	39,748 (99.479)	38,672 (96.687)	35,643 (89.087)	29,989 (74.978)	
Yes	208 (0.521)	1,325 (3.313)	4,366 (10.913)	10,008 (25.022)	
Age, years	32.000 (26.00–39.00)	36.000 (29.00–45.00)	40.000 (32.00–50.00)	43.000 (34.00–53.00)	<0.001
GGT, U/L	17.000 (14.00–22.00)	18.000 (14.00–24.00)	21.000 (16.00–30.00)	25.000 (18.00–39.00)	<0.001
ALT, U/L	13.000 (10.00–17.00)	14.000 (11.00–20.00)	16.000 (12.00–23.00)	19.000 (14.00–27.00)	<0.001
AST, U/L	19.000 (17.00–22.00)	20.000 (17.00–23.00)	21.000 (18.00–25.00)	22.000 (19.00–26.00)	<0.001
TP, g/L	74.33±4.25	73.90±4.24	73.76±4.24	73.87±4.28	<0.001
ALB, g/L	44.89±2.85	44.57±2.84	44.48±2.85	44.58±2.86	<0.001
GLB, g/L	29.5±3.80	29.39±3.84	29.34±3.90	29.35±3.91	<0.001
TB, μmol/L	12.50±5.12	12.19±5.02	12.16±5.02	12.44±5.01	<0.001
BUN, mmol/L	4.000 (3.30–4.80)	4.100 (3.420–4.90)	4.300 (3.60–5.10)	4.450 (3.720–5.300)	<0.001
Scr, mmol/L	69.00 (61.00–81.00)	72.000 (63.00–86.00)	77.000 (65.00–91.00)	84.000 (70.00–95.00)	<0.001
UA, μmol/L	234.0 (194.0–287.0)	248.0 (201.0–310.0)	276.0 (220.0–341.0)	309.0 (247.0–370.0)	<0.001
FPG, mmol/L	4.870 (4.630–5.130)	4.950 (4.700–5.230)	5.030 (4.770–5.350)	5.130 (4.840–5.490)	<0.001
TG, mmol/L	0.850 (0.670–1.090)	0.940 (0.720–1.270)	1.100 (0.810–1.560)	1.330 (0.950–1.930)	<0.001
HDL-c, mmol/L	1.56 (1.340–1.800)	1.48 (1.260–1.730)	1.400 (1.180–1.650)	1.310 (1.120–1.550)	<0.001
LDL-c, mmol/L	2.080 (1.770–2.410)	2.200 (1.870–2.540)	2.310 (1.960–2.640)	2.390 (2.050–2.710)	<0.001

Values are presented as means ± SD or median (Q1–Q3)/*N* (%). NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; GGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; ALB, albumin; GLB, globulin; TB, total bilirubin; BUN, blood urea nitrogen; Scr, creatinine; UA, uric acid; FPG, fasting plasma glucose; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipid cholesterol.

36.000 (29.00–45.00) years, 40.000 (32.00–50.00) years, and 43.000 (34.00–53.00) years ($p < 0.001$). The proportions of males and females were (27.458%; 72.542%), (36.858%; 63.142%), (51.573%; 48.427%), and (66.883%; 33.117%), respectively ($p < 0.001$). The proportions of NAFLD were 0.521%, 3.313%, 10.913%, and 25.022%, respectively ($p < 0.001$). This shows that higher BMI quartiles were associated with increased numbers of NAFLD patients, increased age, and increased proportion of men, while the proportion of women gradually decreased. The results were statistically significant. Increased BMI quartiles were also associated with increased levels of GGT, AST, ALT, BUN, Scr, UA, FPG, TG, and LDL-c ($p < 0.001$), while HDL-c levels decreased ($p < 0.001$). The distributions of TP, ALB, GLB, and TB differed from those of other variables. The highest TP value was observed in Q2, while there was no statistical difference between the Q2 and Q4 groups. The highest values of ALB, GLB, and TB all appeared in the Q1 group with no significant difference in ALB levels between Q2 and Q4, TB levels between the Q1 and Q4, Q2 and Q3 groups, and GLB only differed significantly between the Q1 group and the other three groups.

Association between NAFLD and Potential Risk Factors

Univariate analysis was used to explore the relationship between the potential risk factors for NAFLD and the incidence of NAFLD. Table 2 shows the results. Sex, age, BMI, GGT, AST, ALT, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, TG, and LDL-c were positively correlated with NAFLD, while HDL-c was negatively correlated with NAFLD.

Association between BMI and TG with NAFLD Incidence

BMI and TG were positively correlated with NAFLD in all models, including the crude model, Model I, and Model II ($p < 0.00001$). Table 3 shows the multiple

Table 2. Univariate analysis for NAFLD

Covariate	Statistics	OR (95% CI)	*p* value
Sex			
Female	86,861 (54.302%)	Reference	
Male	73,098 (45.698%)	3.909 (3.767, 4.057)	<0.00001
Age, years	40.214±13.880	1.033 (1.032, 1.034)	<0.00001
BMI	20.975±1.892	2.235 (2.202, 2.268)	<0.00001
GGT, U/L	27.471±30.723	1.018 (1.018, 1.019)	<0.00001
ALT, U/L	19.517±17.548	1.031 (1.030, 1.032)	<0.00001
AST, U/L	22.428±11.331	1.027 (1.026, 1.029)	<0.00001
TP, g/L	73.965±4.256	1.037 (1.033, 1.041)	<0.00001
ALB, g/L	44.628±2.855	1.063 (1.056, 1.069)	<0.00001
GLB, g/L	29.395±3.862	1.012 (1.007, 1.016)	<0.00001
TB, μmol/L	12.322±5.045	1.008 (1.004, 1.012)	0.00005
BUN, mmol/L	4.363±1.278	1.164 (1.150, 1.177)	<0.00001
Scr, mmol/L	77.561±22.238	1.012 (1.011, 1.013)	<0.00001
UA, μmol/L	275.989±86.727	1.008 (1.008, 1.009)	<0.00001
FPG, mmol/L	5.115±0.808	1.658 (1.629, 1.686)	<0.00001
TG, mmol/L	1.256±0.917	2.666 (2.618, 2.716)	<0.00001
HDL-c, mmol/L	1.472±0.356	0.108 (0.102, 0.114)	<0.00001
LDL-c, mmol/L	2.230±0.473	2.587 (2.492, 2.686)	<0.00001

CI, confidence interval; OR, odds ratio.

Table 3. Relationship between BMI and NAFLD

Exposure	Crude model		Model I		Model II	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
BMI	2.235 (2.202, 2.268)	<0.00001	2.042 (2.012, 2.074)	<0.00001	1.788 (1.749, 1.829)	<0.00001
BMI quartile						
Q1	Reference		Reference		Reference	
Q2	6.547 (5.654, 7.582)	<0.00001	5.735 (4.951, 6.643)	<0.00001	4.657 (3.746, 5.79)	<0.00001
Q3	23.408 (20.355, 26.919)	<0.00001	17.429 (15.148, 20.054)	<0.00001	11.248 (9.13, 13.858)	<0.00001
Q4	63.773 (55.55, 73.213)	<0.00001	41.695 (36.29, 47.904)	<0.00001	22.684 (18.445, 27.897)	<0.00001
p for trend	<0.00001		<0.00001		<0.00001	

Crude model: unadjusted. Model I adjusted for sex, age. Model II adjusted for sex, age, GGT, ALT, AST, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, TG, HDL-c, LDL-c.

logistic analysis. The results of the crude model were the same as those of the univariate analysis. The probability of the incidence of NAFLD increased by 1.235 times for every unit increase in BMI (odds ratio [OR] = 2.235, 95% CI 2.202–2.268, $p < 0.00001$). Model I was adjusted for age and sex, and the probability of NAFLD incidence was increased by 1.042 times (OR = 2.042, 95% CI 2.012–2.074, $p < 0.0001$) for each unit increase in BMI. Model II was adjusted for age, sex, GGT, AST, ALT, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, TG, LDL-c, and HDL-c. The probability of NAFLD incidence increased by 0.788 times for each unit increase in BMI (OR = 1.788, 95% CI 1.749–1.829; $p < 0.0001$).

As shown in Table 4, the crude model showed that the probability of NAFLD incidence increased 1.666 times with the increase of one unit of TG (OR = 2.666, 95% CI 2.618–2.716, $p < 0.0001$). Model I was adjusted for age and sex, and the probability of NAFLD incidence increased 1.27 times with each unit increase in TG (OR = 2.270, 95% CI 2.228–2.313, $p < 0.0001$). Model II was adjusted for sex, age, BMI, GGT, AST, ALT, TP, ALB,

Table 4. Relationship between TG and NAFLD

Exposure	Crude model		Model I		Model II	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
TG	2.666 (2.618, 2.716)	<0.00001	2.270 (2.228, 2.313)	<0.00001	1.491 (1.451, 1.532)	<0.00001
TG quartile						
Q1	Reference		Reference		Reference	
Q2	2.314 (2.092, 2.560)	<0.00001	1.949 (1.761, 2.157)	<0.00001	1.379 (1.197, 1.588)	<0.00001
Q3	6.043 (5.513, 6.624)	<0.00001	4.390 (4.000, 4.818)	<0.00001	2.289 (2.009, 2.608)	<0.00001
Q4	26.094 (23.913, 28.473)	<0.00001	16.48 (15.08, 18.01)	<0.00001	5.070 (4.456, 5.768)	<0.00001
p for trend	<0.00001		<0.00001		<0.00001	

Crude model: unadjusted. Model I adjusted for sex, age. Model II adjusted for sex, age, BMI, GGT, ALT, AST, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, HDL-c, LDL-c.

GLB, TB, BUN, Scr, UA, FPG, LDL-c, and HDL-c, and the probability of NAFLD increased 0.491 times with each unit increase in TG (OR = 1.491, 95% CI 1.451–1.532, $p < 0.0001$).

The BMI and TG levels of the subjects were analyzed as continuous variables. The *p* value for the trend was<0.00001 in each model, indicating that the incidence of NAFLD increased significantly with the increase of BMI and TG by one to four quantiles.

Multivariate Linear Regression between BMI and TG

As shown in Table 5, BMI and TG were significantly positively correlated. Multivariate linear regression was used to obtain the crude model. For every 1 kg/m^2 increase in BMI, the TG level increased by 0.138 mmol/L (β = 0.138, 95% CI 0.135–0.140, $p < 0.0001$). Model I was adjusted for age and sex and showed that with every 1 kg/m^2 increase in BMI, TG levels increased by 0.095 mmol/L (β = 0.095, 95% CI 0.092–0.097, $p < 0.0001$). Model II was adjusted for age, sex, NAFLD, GGT, AST, ALT, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, LDL-c, and HDL-c, and showed that with every 1 kg/m^2 increase in BMI, the TG level increased by 0.027 mmol/L (β = 0.027, 95% CI 0.023–0.030, $p < 0.00001$). When BMI increased by a quartile, the TG level increased significantly (*p* for trend <0.00001).

Mediated Effect

Mediation analysis was performed to explore whether and to what extent the relationship between BMI and NAFLD was mediated by TG in Chinese people with BMI <24 kg/m^2 (as shown in Fig. 2, after adjusting for sex, age, GGT, AST, ALT, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, LDL-c, and HDL-c. The natural direct effect refers to the direct effect of BMI on NAFLD (β = 0.1081, 95% CI 0.1041–0.1115 $p < 0.0001$), while the NIE refers to the effect of BMI on NAFLD through the mediation of TG (β = 0.0033, 95% CI 0.0029–0.0038 $p < 0.00001$). PM represents the NIE divided by the total effect and showed that an impact of about 2.98% BMI on NAFLD in the population with BMI <24 kg/m^2 was mediated by TG (β = 0.0298, $p < 0.0001$, 95% CI 0.0257–0.0343).

Discussion

The study investigated a Chinese population with BMI <24 kg/m^2. This cross-sectional study enrolled 159,959 individuals with normal LDL-c levels and BMI <24 kg/m^2, of whom 15,907 (9.94%) had been diagnosed with NAFLD. The results showed that in the population with BMI <24 kg/m^2, for every unit increase in BMI and TG, the probability of NAFLD incidence increased 0.788 and 0.491 times, respectively, with TG mediating 2.98% of the positive correlation between BMI and non-obese NAFLD.

As NAFLD is increasingly diagnosed in people with normal BMI, there has been an increase in research focused on NAFLD in non-obese individuals. In such patients, the relationship between BMI, TG levels, and NAFLD is still controversial. Xing et al. [16] found that in a group with BMI <24 kg/m^2, BMI was a risk factor for NAFLD while TG was not. Moreover, TG showed no significant mediation of the correlation between BMI and NAFLD. Significant mediating effects were observed only in the group with BMI over 24 kg/m^2. Ren et al. [22] found that there was no significant difference in TG levels between individuals with BMI <24 kg/m^2 and overweight

Table 5. Relationship between BMI and TG

Exposure	Crude model		Model I		Model II	
	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value
BMI	0.138 (0.135, 0.140)	<0.00001	0.095 (0.092, 0.097)	<0.00001	0.027 (0.023, 0.030)	<0.00001
BMI quartile						
Q1	Reference		Reference		Reference	
Q2	0.162 (0.150, 0.174)	<0.00001	0.101 (0.089, 0.113)	<0.00001	0.037 (0.020, 0.053)	<0.00001
Q3	0.397 (0.385, 0.410)	<0.00001	0.257 (0.245, 0.269)	<0.00001	0.081 (0.064, 0.098)	<0.00001
Q4	0.697 (0.684, 0.709)	<0.00001	0.481 (0.468, 0.494)	<0.00001	0.132 (0.114, 0.150)	<0.00001
p for trend	<0.00001		<0.00001		<0.00001	

Crude model: unadjusted. Model I adjusted for sex, age. Model II adjusted for sex, age, NAFLD, GGT, ALT, AST, TP, ALB, GLB, TB, BUN, Scr, UA, FPG, HDL-c, LDL-c.

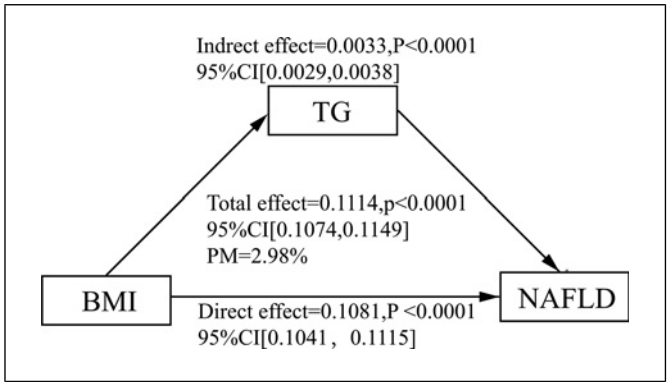

Fig. 2. Mediation analysis adjusting variables: sex, age, GGT, ALT, AST, TP, ALB, FPG, TB, BUN, Scr, UA, FPG, HDL-c, LDL-c.

or obese NAFLD patients, although the BMI <24 kg/m^2 group had higher levels of visceral fat. Our cross-sectional study found that, when all confounding factors were adjusted, the BMI and TG levels of non-overweight Chinese people with BMI <24 kg/m^2 and normal LDL-c levels were positively correlated with the incidence of NAFLD. Consistent with our findings, a cohort study of male Korean workers found that an increase of 2.3 kg in the body mass of participants with BMI between 18.5 kg/m^2 and 22.9 kg/m^2 was correlated with fatty liver, and weight gain was an independent predictor of lean NAFLD [23]. Another study showed that BMI and TG were still pathogenic factors in NAFLD patients with BMI <25 kg/m^2, and TG levels of 1.7–2.25 mmol/L were closely associated with NAFLD [15]. A community prospective study by Wong et al. [24] found that in patients with BMI <25 kg/m^2, reductions in TG level, weight, and waist circumference were independent influencing factors for remission in "lean" NAFLD patients. We found that BMI still had a direct effect on NAFLD in about 10.81% of non-overweight people. In the population with BMI <24 kg/m^2, the correlation between the highest quartile of BMI and NAFLD was 22.684 times greater than that of the lowest quartile ($p < 0.00001$). Similar to our findings, other studies have shown that due to genetic polymorphisms in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene in Asian populations, people with fluctuations in BMI that do not lead to weight gain and obesity are still prone to metabolic disorders and NAFLD [10]. Moderate weight gain in individuals with normal BMI can lead to metabolic disorders, which usually manifest as increased visceral fat [25]. Visceral adiposity can lead to lean NAFLD [26]. In other words, small changes in body mass can increase the risk of fatty liver [27]. The visceral obesity index of non-obese NAFLD patients can be higher than that of overweight or obese NAFLD patients [22], indicating that patients with lower BMI may have a reduced metabolic capacity for minor weight gain than patients with higher BMI [28]. As a result, compared with obese NAFLD patients, dietary and lifestyle interventions such as reducing sedentary habits are more suitable for lean or non-obese NAFLD patients [29]. Whether used for the prevention or treatment of non-obese NAFLD, diet and exercise strategies such as the consumption of fish rich in n-3 PUFAs and increasing the frequency of exercise are very important [30]. An RCT study has suggested that non-obese patients can still reduce weight and TG levels through lifestyle intervention and thus alleviate NAFLD. In terms of prognosis, non-obese patients are more likely to maintain normal liver function than obese patients [24].

The analysis of clinical mediation in this study also showed that TG mediated the association between BMI and non-obese NAFLD by about 2.98%. This appears to support the view that TG may play a role in this association. TG levels are known to increase with increased BMI and NAFLD severity. A key factor in the development of NAFLD is a high TG level [31]. A cohort study based on the UK Biobank found that hypertriglyceridemia was an important marker for predicting NAFLD in patients with BMI <25 kg/m^2 [32]. A moderate rise in BMI in people with lower BMI may lead to metabolic disorders, thus causing an increase in visceral fat, which is usually accompanied by abnormal blood lipid levels, resulting in an accumulation of TG in the liver and potentially leading to hepatocyte steatosis and, ultimately, NAFLD [22]. TG levels and free fatty acids (FFAs) are negatively correlated with insulin sensitivity [33], as FFAs interfere with the binding of insulin to its receptor, thus reducing the effect of insulin and even leading to IR. IR is the pathophysiological basis of metabolic disorder, further promotes the synthesis of FFAs in liver cells, and reduces very low-density lipoprotein output, forming a vicious circle and aggravating NAFLD [34, 35]. The accumulation of TG is caused by the supply of fatty acids (FAs) through various pathways. When TG accumulates, FA is also excessive, and mitochondrial fatty acid oxidation and reactive oxygen species (ROS) levels are increased due to IR, leading to a redox imbalance and the progression of NAFLD into irreversible diseases such as NASH and cirrhosis [12, 31]. Therefore, we believe that in non-obese NAFLD patients, TG may also indirectly regulate the association between moderately increased BMI and NAFLD in non-obese individuals by promoting IR-induced redox imbalances. In addition, the mediating role of TG may also occur through various mechanisms. For example, Peng et al. [36] found that increased expression of GP73 protein in the liver was associated with symptoms related to "lean" NAFLD, such as visceral obesity, which can increase the excessive accumulation of TG and cholesterol in the liver, leading to abnormal liver metabolism and IR. In terms of genetic factors, GM4951, an immunity-related GTPase, can regulate liver lipid metabolism. Experiments show that a deficiency in GM4951 can lead to "lean" NAFLD [37]. Our results showed that TG only mediated about 2.98% of the positive correlation between BMI and non-obese NAFLD, indicating the involvement of other mechanisms mediating BMI and non-obese NAFLD. The direct effect of BMI on lean NAFLD in non-obese people is only about 10.81%, indicating the presence of additional factors. For example, NAFLD patients often consume high levels of sugar and fat while their diet lacks n-3 PUFAs, leading to reduced activity of the PPAR-α transcription factor; this lower PPAR-α activity will, in turn, promote increased inflammatory responses and mitochondrial dysfunction [13].

Our research has a number of advantages. First, it is a large-scale study based on 159,959 participants. Second, we included non-overweight and non-obese (BMI <24 kg/m^2) people with normal LDL-c levels as research subjects, according to the Chinese BMI index classification. The results can thus better reflect the situation with non-obesity-associated NAFLD in non-overweight people in China and can also provide theoretical guidance for improving the condition of non-obese NAFLD patients. Finally, we used a variety of statistical methods, including univariate analysis, multiple logistic analysis, multivariate linear regression, mediation analysis, and p for trend.

This study has some limitations. First, it was a single-center study, and the population was composed entirely of patients from Wenzhou People's Hospital in China, and thus may lack broad applicability. Second, ultrasound was used instead of the gold standard of liver biopsy for the diagnosis of NAFLD. This is because liver biopsy is invasive and does not meet ethical requirements. Ultrasound has the advantages of safety and economy and is suitable for large-scale epidemiological research. Third, the study did not take into account the life history of the patients, such as lifestyle and eating habits, history of diseases such as hypertension and diabetes, and laboratory data such as PT, alkaline phosphatase, and total cholesterol, and thus was unable to control for these potential confounding factors. Fourthly, the original research did not measure visceral obesity either by anthropometric measures, i.e., waist circumference, or by the US measure of visceral fat thickness, so we could not explore the relationship between visceral obesity and non-obese NAFLD. Finally, this was a cross-sectional study, and thus causal relationships cannot be drawn. In the future, a large, multicenter cohort study would be able to further explore the relationships between non-obese NAFLD, BMI, and TG.

Conclusions

In a Chinese population with BMI <24 kg/m^2 and normal LDL-c levels, BMI and TG were found to be independent predictors of NAFLD. The direct effect of BMI on non-obese NAFLD was 10.41%. The TG level was found to partially mediate the association. This suggests

that non-obese NAFLD can be alleviated by weight loss and that TG should be considered as a therapeutic target. However, due to the relatively small mediation effect and PM, more significant mechanisms are expected to be found.

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Statement of Ethics

Ethical approval and consent were not required as this study was based on publicly available data.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

X.H. and C.W. put forward the main idea of the article. X.H. was responsible for data analysis and manuscript writing. J.K. Y.Z., and Ya.Z. were responsible for data analysis, data collection, and final proofreading. Z.M. and C.W. revised this paper. X.H. J.K., H.Z., Y.Z., Ya.Z., and C.W. read and approved the final version of this paper and agree with the order of the presentation of the authors.

Data Availability Statement

The data can be downloaded from DATADRYAD database. Further inquiries can be directed to the corresponding author.

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