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ORIGINAL RESEARCH

The Mediation Role of Insulin Resistance and Chronic Systemic Inflammation in the Association Between Obesity and NAFLD: Two Cross-Sectional and a Mendelian Randomization Study

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Purpose: We aimed to identify the association between obesity and nonalcoholic fatty liver disease (NAFLD) and to quantify the mediating effects of insulin resistance (IR) and chronic inflammation through observational studies and Mendelian randomization (MR).

Patients and Methods: In the current study, three IR-related indicators and three indicators of inflammation were included. The individual and combined mediated effects of IR and inflammation in the association between obesity and NAFLD were investigated in two cross-sectional studies, the Fuqing Cohort from China and the National Health and Nutrition Examination Survey (NHANES). Total, direct, and indirect effects were estimated through direct counterfactual imputation estimation, and the proportion of mediating effects was calculated. We applied a two-step MR to determine the causal mediating role of IR and chronic inflammation in the pathway between obesity and NAFLD by using single nucleotide polymorphisms as instrumental variables to predict obesity, IR, and inflammation genetically.

Results: In the Fuqing Cohort, all obese phenotypes were associated with an elevated NAFLD risk. Moreover, indicators of IR such as homeostatic model assessment of insulin resistance (HOMA-IR) and indicators of inflammation such as C-reactive protein (CRP) were significantly and positively associated with NAFLD risk. Individuals with obesity had significantly higher levels of IR and inflammation indicators compared to non-obese individuals. The indirect proportions of insulin and HOMA-IR accounted for 50.97–66.72% in the associations between obese phenotypes and NAFLD risk, while the proportions of inflammation indicators were < 14%. Similar results were observed in the NHANES analysis. In the MR analysis, the indirect effects of HOMA-IR and CRP were statistically significant with a greater mediated proportion explained by HOMA-IR than CRP.

Conclusion: Through two population-based studies and MR, we found the causal mediation roles of IR and inflammation in the association between obesity and NAFLD, in which HOMA-IR and CRP showed stable, significant mediation effects. Furthermore, HOMA-IR showed a higher mediation effect than CRP. We emphasize the vital role of HOMA-IR in NAFLD monitoring.

Keywords: NAFLD, insulin resistance, inflammation, mediation analysis, Mendelian randomization

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Introduction

Since last century, the prevalence of excess body weight has increased rapidly, affecting more than 2 billion adults, and the prevalence of overweight and obesity is estimated to double in the next 10 years worldwide.[1] Furthermore, it has been reported that obesity contributes to more than 40% of metabolism-related mortality globally, leading to the largest burden of metabolic diseases.[2–4] Nonalcoholic fatty liver disease (NAFLD) is a metabolic disease of intrahepatic fat accumulation, calling for special attention for its alarming increase over the past two decades.[2–4] Globally, NAFLD has become the most common liver disease with a prevalence of approximately 25%.[5] In our study population, its prevalence was as high as 32.4%, showing a heavy disease burden in Southeast China.[6,7] Previous studies have shown that obesity is an important risk factor for NAFLD,[8–11] thus the rising epidemic of obesity urges us to understand the pathogenic pathways of NAFLD to implement effective prevention, monitoring, and treatment strategies.[4]

Excess body weight is characterized by unfavorable body composition with excess adipose tissues,[12] which deposit as ectopic fat and generate lipotoxicity inside the cells of various organs, such as the liver, muscle, and pancreas. The lipotoxicity in sustained obesity then leads to organelle dysfunction, which in turn causes insulin resistance (IR) and chronic low-grade systemic inflammation, creating the circumstances to develop clinical conditions.[13]

With the deepening of research on fatty liver disease, a new nomenclature—metabolic dysfunction–associated steatotic liver disease (MASLD)—has been proposed to emphasize the critical role of metabolic dysfunction in the disease's development and progression.[14] Fatty liver disease has been recognized as a systemic metabolic disease frequently accompanied by IR and systemic inflammation.[15] The liver is more sensitive to insulin impairment than other organs, thus IR shows a predictive value for the development of hepatic steatosis, which is the most common manifestation of NAFLD.[13,16] Besides, chronic inflammation has been reported to initiate and/or maintain multiorgan dysfunction and increase the susceptibility to various metabolic disorders.[17,18] However, whether IR and chronic inflammation are causal mediators in the association between obesity and NAFLD, and how much the mediated effects of IR and chronic inflammation contribute in the association between obesity and NAFLD have not yet been elucidated.

The gold standard to assess IR is the hyperinsulinemic clamp, a time-consuming and invasive procedure, which is often impractical to apply in epidemiological settings.[19] As an alternative, homeostatic modeling assessments provide convenient and validated indicators: homeostatic model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment of beta-cell function index (HOMA-beta), capable of reflecting the level of IR as well as beta cell function, respectively.[19] For inflammation assessment, one can utilize C reactive protein (CRP), white blood cell count (WBC), and lymphocyte count (LYMPH) indicators to provide a more comprehensive description of their effects, based on previous studies.[6]

Recently, Mendelian randomization (MR) has emerged as a powerful tool for causal inference, utilizing single nucleotide polymorphisms (SNPs) as instrumental variables (IVs).[20] Since genetic variants are randomly assigned during meiosis, they are typically not influenced by environmental factors and precede the emergence of disease phenotypes. This inherent randomness mimics the randomization process in randomized controlled trials, significantly reducing the risk of confounding and reverse causation when randomized controlled trials are not feasible, and providing robust causal evidence for the relationships between exposures and outcomes in the context of complex metabolic diseases.[21]

Thus, in the current study, we investigated the mediation effects of IR and systemic chronic inflammation in the association of obesity-NAFLD in the Fuqing cohort study, conducted in a community-welling population from southeast China, and the National Health and Nutrition Examination Survey (NHANES), respectively. Then, the MR approach was adopted to assess the causal association of obesity, IR or inflammation, and NAFLD, which could help infer the causal mediation of IR and inflammation in the association of obesity and NAFLD.

Methods

Overall Study Design

This study utilized population-based cross-sectional data to estimate the mediating effects of IR and inflammation on the association between obesity and NAFLD and validated it in the NHANES 2007–2018 data. Given that cross-sectional

studies cannot effectively infer causality, we employed a two-sample MR analysis to assess potentially causal associations.

Fuqing Cohort Study

Study Design and Participants

We are conducting a prospective cohort study in Fuqing City - Fuqing Cohort Study, in Southeast China, in which native residents aged 35–75 years were recruited.[22] In the current study, we included 7662 participants from Gaoshan Town with the completion of a baseline survey from July 2020 to June 2021.[6,7] After excluding those with incomplete information, outliers, and excessive alcohol consumption,[23] a total of 6036 participants were included in the current study (Figure S1).

The Ethics Review Committee of Fujian Medical University approved the study protocol (approval number, [2017-–07] and [2020–58]) before data collection. Written informed consents were obtained from all participants.

Data Collection

All participants were instructed to complete a structured-questionnaire and physical examinations, which has been described in previous studies.[24,25]

Obesity

General obesity was defined by body mass index (BMI, obesity_BMI: ≥ 28.0 kg/m^2).[26] Central obesity was defined by waist circumference (WC, obesity_WC: men ≥ 90 cm, women ≥ 85 cm),[27] waist-to-hip ratio (WHR, obesity_WHR: men ≥ 0.9, women ≥ 0.85),[28] and waist-to-height ratio (WHtR, obesity_WHtR: ≥ 0.5),[28] respectively.

IR, Insulin Sensitivity and Inflammation Indicators

The formulae for calculating the indicators of IR and insulin sensitivity are listed in the following:

HOMA-IR: [fasting blood glucose (mmol/L) × fasting insulin (mU/L)]/22.5;[29]

HOMA-beta: 20 × fasting insulin (mU/L) / [fasting blood glucose (mmol/L) - 3.5]%;[29]

Inflammatory indicators include CRP, WBC, and LYMPH in the current study.

NAFLD Definition in Fuqing Cohort

Ultrasonography was performed on all participants by experienced sonographers who were unaware of the clinical or laboratory data of the participants using ultrasound scanners (Hitachi Aloka Medical, ProSounda α7, Japan). According to the criteria published by the Fatty Liver Disease Study Group of the Chinese Liver Disease Association, the diagnosis of NAFLD was based on abdominal ultrasound images, and those with mild fatty liver and above were diagnosed as NAFLD patients.[30]

Covariates

Current smokers were defined as those with an average use of cigarettes ≥ 1 per day for at least 6 months. Current alcohol drinkers were those who drank ≥ 1 times per week in the past year. The international physical activity questionnaire (short form) was used to calculate physical metabolic equivalent (MET/day),[31] and physical activity was classified into light, moderate, and heavy according to the tertiles of MET.

Statistical Analysis

Continuous data are summarized as mean ± standardized deviation (SD). Student's *t*-test or Mann–Whitney *U*-test was conducted to compare the differences between different groups for continuous variables. Categorical data are shown as proportions, and the Chi-squared test was used to compare the differences between groups.

Multivariable logistic regression models were constructed to control potential confounding effects. Regression model 1 was a rough model without adjustment. Model 2 was adjusted for sex and age. Model 3 was further adjusted for educational level, current alcohol drinking, current smoking, and physical activity in addition to those included in model 2. The odds ratios (ORs) and 95% confidence intervals (CIs) for IR and inflammatory markers were calculated. Multiple linear regression models were used to assess the relationship between insulin, HOMA-IR, HOMA-beta, inflammatory

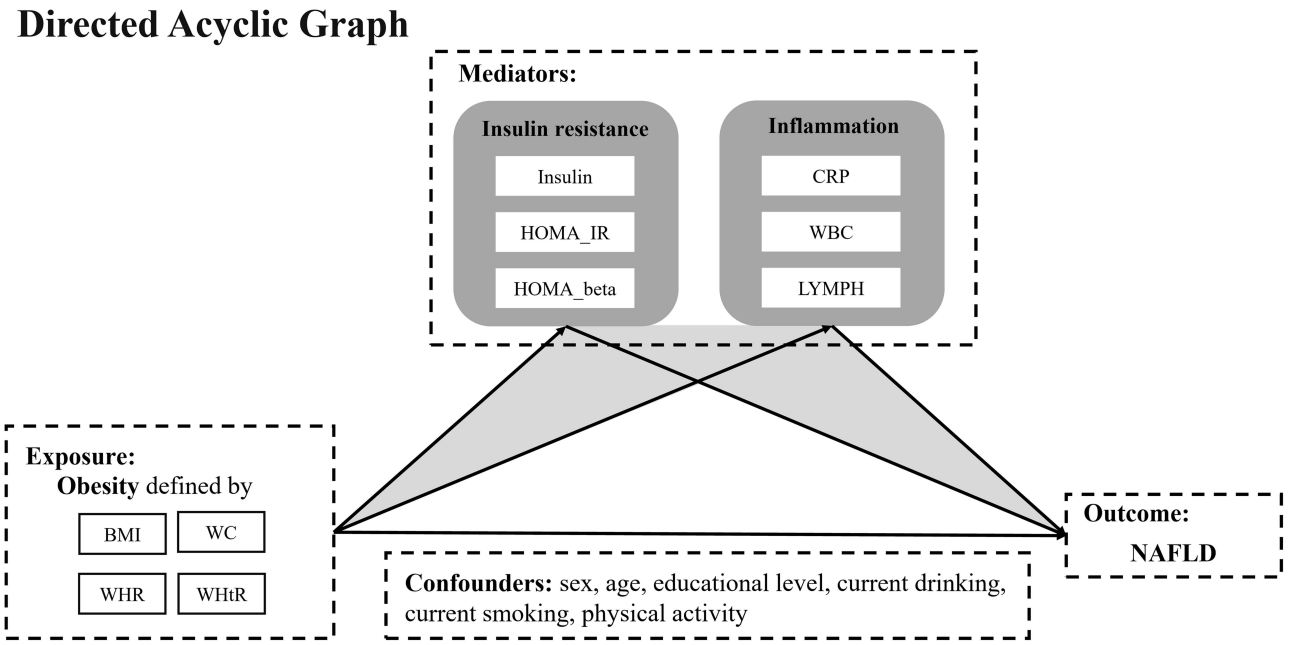

Figure 1 The directed acyclic graph for current study.
Abbreviations: BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-beta, homeostatic model assessment of beta-cell function index; CRP, C reactive protein; WBC, white blood cell count; LYMPH, lymphocyte count; NAFLD, nonalcoholic fatty liver disease.

indicators, and obesity. Each model was adjusted for potential confounders, including age, sex, educational level, current alcohol drinking, current smoking, and physical activity.

To explore the potential mediating effects of insulin, HOMA-IR, HOMA-beta, and inflammatory indicators in the relationship between obesity and NAFLD, we performed mediation analysis using a regression-based approach.[32] The directed acyclic graph is presented in Figure 1. Considering the different units of each potential mediator, we grouped all mediators according to quartiles in order to improve the comparability of results (Q1: ≤ 25th percentile, Q2: 25th percentile - ≤ 50th percentile, Q3: 50th percentile - ≤ 75th percentile, Q4: >75th percentile). The "CMAverse"[33] package in R was used to estimate the OR of total effect, direct effect, and indirect effect through direct counterfactual imputation estimation. The adjusted variables included age, sex, educational level, current alcohol drinking, current smoking, and physical activity.

Sensitivity Analysis

The triglyceride-glucose index (TyG: ln [fasting triglycerides (mg/dl) ×fasting blood glucose (mg/dl)]/2) is an emerging accessible tool for assessment of IR because of its consistency with the high insulin-glucose clamp test.[34,35] The systemic inflammation response index (SIRI: monocyte × neutrophil/lymphocyte), is an effective biomarker in reflecting the dynamics of inflammation and immune status based on monocyte, neutrophil, and lymphocyte counts.[36,37] Thus, sensitivity analyses were performed to confirm the potential mediating effects using the above two indexes as mediators to proxy for IR and inflammation levels.

Diabetes was strongly associated with obesity, IR, and inflammation,[38] so we performed additional sensitivity analyses to include diabetes status in the adjusted model.

NHANES

Study Design and Participants

The NHANES is a national, cross-sectional, multistage probability sampling survey that provides representative samples of the non-institutionalized US resident population. All data collected from NHANES participants have been approved by the Ethics Review Board of the National Center for Health Statistics (NCHS) (more information about NHANES can

be obtained at: https://www.cdc.gov/nchs/nhanes/). In the current study, we included 22,567 participants aged 35–75 years from the 2007–2018 NHANES dataset. Exclusion criteria were as follows: (1) missing values for sociodemographic characteristics and body fat distribution (n=2674); (2) missing components on United States Fatty Liver Index (USFLI) calculation (n=11,124); (3) excessive alcohol consumption based on questionnaire responses (n=918); (4) missing values on hepatitis B virus (HBV) or hepatitis C virus (HCV) infection status, or individuals with HBV/HCV infection (n=229); (5) missing values for inflammation indicators (n=2434); and (6) pregnant women (n=21). After applying these exclusion criteria, a total of 5167 participants were included in our final analysis.

Definition of NAFLD in NHANES

The USFLI was utilized to diagnose NAFLD in this study due to the absence of abdominal ultrasound data. The USFLI is an improved version of the Fatty Liver Index that is better suited for the US population. It uses abdominal ultrasound results as the gold standard and has an area under the receiver operating characteristic curve of 0.80 (95% CI: 0.77–0.83).[39] Participants were classified as having NAFLD if their USFLI was ≥ 30, according to the recommended cut-off value:[40]

$$\text{USFLI} = e^{(0.3458*\text{Mexican American}-0.8073*\text{non-Hispanic Black}+0.0093*\text{age}+0.6151*\ln(\text{GGT})+0.0249*\text{waist circumference}+1.1792*\ln(\text{insulin})+0.8242*\ln(\text{glucose})-14.7812)}/(1 + e^{(0.3458*\text{Mexican American}-0.8073*\text{non-Hispanic Black}+0.0093*\text{age}+0.6151*\ln(\text{GGT})+0.0249*\text{waist circumference}+1.1792*\ln(\text{insulin})+0.8242*\ln(\text{glucose})-14.7812)}) * 100.$$

The values for "non-Hispanic Black" and "Mexican American" are assigned as "1" if the participant belongs to that ethnicity and "0" if they do not belong to that ethnicity.

Definition of Obesity Phenotypes

Due to the absence of hip circumference data in the NHANES database, our analysis included only the following obesity phenotypes: obesity_BMI (≥ 30.0 kg/m^2), obesity_WC (WC > 102 cm for men or > 88 cm for women), and obesity_WHtR (WHtR ≥ 0.5).[41]

Covariates

Data on age, sex, race, education, smoking status, alcohol consumption, and physical activity were collected using self-reported questionnaires in the Mobile Examination Center. Current alcohol drinkers were defined as individuals who consumed alcohol at least once per week over the past year. Metabolic equivalent scores were calculated using the NHANES reference tables and categorized into tertiles.

Statistical Analysis

All analyses were conducted while accounting for the complex survey design utilizing the appropriate primary sampling unit, pseudo-strata, and sampling weights unless otherwise specified. Survey-weighted logistic regressions were employed to investigate the associations of obesity phenotypes, insulin-related indicators, and inflammation indicators with NAFLD after adjusting for confounders (age, sex, race, educational level, smoking status, current alcohol consumption, and physical activity). The "Survey" package in R was utilized for complex survey designs. Following VanderWeele's recommendations, a mediation analysis was conducted using the "CMAverse" package, consistent with the methodology previously described for the Fuqing cohort.[42]

MR Study

In the current study, we further used two-sample MR to assess the association between obesity and NAFLD. In addition, a two-step MR design was used to investigate whether IR and inflammation traits explained the effect of obesity on NAFLD risk. In step 1, we first performed the two-sample MR to determine the causal effects of different subtypes of obesity on potential mediators (IR and inflammation), which utilized SNPs as IVs to genetically predict obesity. In step 2, genetic IVs associated with the potential mediators were used to estimate the causal effect of the potential mediator on NAFLD and determine the causal role in the mediating pathway between obesity and NAFLD (Figure S2).

Data Sources of Exposures, Mediators, and Outcome

Summary-level data of genome-wide association studies (GWAS) for exposures, mediators, and outcome were derived from the IEU Open GWAS project (https://gwas.mrcieu.ac.uk/).

The overlapping of participants in the two-sample MR may lead to an inflated type 1 error rate due to weak instrumental bias. Accordingly, to minimize the participant's overlap, we selected exposure, mediation, and outcome datasets separately from different consortiums or cohorts. Genetic instruments for each obesity phenotype were selected as IVs from the GWAS dataset of the Genetic Investigation of Anthropometric Traits (GIANT) Consortium. Genetic instruments for IR and inflammation indicators were obtained from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) and UK Biobank, respectively. For NAFLD, we selected instruments from a Finnish cohort-based dataset that contained 894 cases and 217,898 controls. Detailed information about the data sources and sample sizes used in the current study is provided in Table S1. All summary-level data have been made publicly available, and ethical approval was obtained in the original studies.

Instrumental Variable Selection

To conduct effective causal inference using MR, three key assumptions must be met: (1) the IVs are strongly associated with the exposure, (2) the IVs are not associated with any potential confounder, and (3) the IVs do not affect outcome independently of exposure. To meet these assumptions, we undertook a series of methodological steps. First, we extracted genome-wide significant SNPs to proxy for the effect of each trait ($P < 5\times10^{-8}$). Because few SNPs were screened out for IR indicators (insulin, HOMA-IR, and HOMA-beta) at the threshold of $P < 5\times10^{-8}$, we thus applied a more liberal threshold for the candidate IVs selection ($P < 5\times10^{-5}$). Second, to identify independent SNPs, we conducted linkage disequilibrium (LD) pruning for each trait. This analysis was performed using the One Thousand Genomes LD reference panel, with a particular focus on individuals of European descent. We applied pairwise LD thresholds from the original GWAS for each trait, with SNPs for each trait adhering to a LD cut-off of $R^2 > 0.001$ within a window of 10,000 kb. Then, to ensure accurate causal estimates in MR analysis, we used the "TwoSampleMR" R package to harmonize alleles in the GWAS summary statistics. This process aligned the effects of SNPs on both exposure and outcome to the same allele and excluded ambiguous palindromic SNPs.

Statistical Analysis

An inverse variance weighted (IVW) method was used as our main method, which combined the Wald ratio estimates of each SNP into one causal estimate for each risk factor using the random-effects meta-analysis approach.[43] This method allows for robust causal inference even in the presence of horizontal pleiotropy. The results were expressed as ORs on NAFLD risk per one standard deviation unit increase in BMI, WC, or WHR. In the two-step MR analysis, the total effect (β0) was first obtained between each obesity indicator and NAFLD; then the effect of obesity on each mediator (β1) and the effect of each mediator on NAFLD (β2) was estimated. The indirect effect was calculated by multiplying the effect of obesity on the mediator and the effect of the mediator on NAFLD (β1*β2). Standard errors for the indirect effects were derived by using the Delta method. The proportion of mediated effect by each mediator was estimated through dividing the indirect effect by the total effect (β1*β2/β0).

We used the intercept term of the MR Egger to test for the potential presence of horizontal pleiotropy, where deviation from zero ($P<0.05$) was considered to provide evidence for the presence of directional pleiotropic bias. When potential pleiotropy was found, we further used MR-Egger regression for sensitivity analysis. We applied Cochran's Q heterogeneity statistic to assess the heterogeneity between instruments. The leave-one-SNP-out sensitivity test was conducted to examine the influence of individual variants on the observed associations. We further performed the MR Steiger test to check the potential reverse causal impact.[44] All statistical analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA), and R, version 4.3.0. Statistical significance was considered at two-tailed $P < 0.05$.

Results

Fuqing Cohort Study

The Demographic Characteristics of Participants in the Fuqing Cohort Study

A total of 6036 participants were enrolled in the current study. According to BMI categories, the prevalence of obesity in males was significantly lower compared with that in females (9.9% vs 12.2%). Similar patterns were observed in central obesity defined by WC, WHR, or WHtR. The prevalence of NAFLD was 35.0% in the whole population (Table S2).

Associations of Obesity, IR, Inflammation, and NAFLD in the Fuqing Cohort Study

The baseline characteristics of the participants stratified by NAFLD are presented in Table 1. Participants with NAFLD had a significantly higher prevalence of obesity/central obesity. The association between obesity and NAFLD is summarized in Table 2. In multivariable logistic regression models, the adjusted ORs for NAFLD of all obesity phenotypes were higher than 5.00.

Table 2 also demonstrates the association between IR, inflammation, and NAFLD. Significant associations were observed between all indicators for IR or inflammation and NAFLD across different models. The adjusted ORs of six indicators gradually increased from the second- (Q2) to the highest-level (Q4) group, compared to the lowest-level group (Q1). The ORs for the Q4 group for insulin and HOMA-IR were 18.37 (95% CI: 15.06–22.40) and 24.47 (95% CI: 19.85–30.16), higher than the ORs of other indicators in fully adjusted models.

The obese population defined by any criteria had higher levels of insulin, HOMA-IR, HOMA-beta, CRP, WBC, and LYMPH (Figure S3). In multivariable linear regression models, obesity was associated with higher levels of insulin,

Table 1 The Clinico-Demographic Characteristics of the Population, Overall and Stratified by Presence of NAFLD in Fuqing Cohort Study

	Total	NAFLD		*P*
		No (n=3924)	Yes (n=2112)	
Sex				0.226
Male	1969(32.6)	1259(32.1)	710(33.6)	
Female	4067(67.4)	2665(67.9)	1402(66.4)	
Age, years				<0.001
35–44	748(12.4)	559(14.2)	189(8.9)	
45–54	1587(26.3)	1063(27.1)	524(24.8)	
55–64	2036(33.7)	1259(32.1)	777(36.8)	
65–75	1665(27.6)	1043(26.6)	622(29.5)	
Educational level, years				0.140
0	2086(34.6)	1319(33.6)	767(36.3)	
1–6	2060(34.1)	1352(34.5)	708(33.5)	
7–9	1352(22.4)	905(23.1)	447(21.2)	
>9	538(8.9)	348(8.9)	190(9.0)	
Current occupation				0.128
Farmer or unemployment	4433(73.4)	2864(73.0)	1569(74.3)	
Worker	591(9.8)	410(10.4)	181(8.6)	
Sales or service	369(6.1)	246(6.3)	123(5.8)	
Official job	584(9.7)	368(9.4)	216(10.2)	
Other	59(1.0)	36(0.9)	23(1.1)	
Current alcohol drinking	337(5.6)	3696(94.2)	2003(94.8)	0.295
Current smoking	939(15.6)	625(15.9)	314(14.9)	0.278
Physical activity				0.236
Low	1999(33.1)	1302(33.2)	697(33.0)	
Moderate	2038(33.8)	1298(33.1)	740(35.0)	
High	1999(33.1)	1324(33.7)	675(32.0)	
Obesity phenotypes				
Obesity_BMI	691(11.5)	149(3.8)	542(25.7)	<0.001
Obesity_WC	2081(34.5)	768(19.6)	1313(62.2)	<0.001
Obesity_WHR	3484(57.7)	1761(44.9)	1723(81.6)	<0.001
Obesity_WHtR	3818(63.3)	1923(49.0)	1895(89.7)	<0.001

Notes: All data are shown as number (percentage).
Abbreviations: NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 2 The Association of Obesity, Indicators of Insulin Resistance, and Indicators of Inflammation With NAFLD in Fuqing Cohort Study

	NAFLD		*P*	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	No (n=3924)	Yes (n=2112)				
Obesity phenotypes						
Obesity_BMI	149(3.8)	542(25.7)	<0.001	8.75 (7.23–10.58)	8.77 (7.24–10.62)	8.75 (7.22–10.59)
Obesity_WC	768(19.6)	1313(62.2)	<0.001	6.75 (6.00–7.60)	6.78 (6.01–7.65)	6.78 (6.01–7.65)
Obesity_WHR	1761(44.9)	1723(81.6)	<0.001	5.44 (4.79–6.18)	5.54 (4.86–6.31)	5.57 (4.89–6.35)
Obesity_WHtR	1923(49.0)	1895(89.7)	<0.001	9.09 (7.79–10.60)	9.29 (7.95–10.87)	9.41 (8.04–11.01)
Indicators on IR						
Insulin	6.66±3.59	11.18±6.53	<0.001	3.52 (3.23–3.83)	3.82 (3.50–4.17)	3.82 (3.50–4.18)
Q1	1341(34.2)	175(8.3)	<0.001	Reference	Reference	Reference
Q2	1163(29.6)	340(16.1)		2.24 (1.84–2.73)	2.51 (2.06–3.08)	2.52 (2.06–3.09)
Q3	908(23.1)	601(28.5)		5.07 (4.20–6.12)	5.85 (4.82–7.10)	5.87 (4.83–7.12)
Q4	512(13.0)	996(47.2)		14.91 (12.33–18.03)	18.31 (15.02–22.32)	18.37 (15.06–22.40)
HOMA-IR	1.51±0.96	2.93±2.44	<0.001	5.41 (4.84–6.04)	5.66 (5.05–6.34)	5.65 (5.05–6.33)
Q1	1363(34.7)	146(6.9)	<0.001	Reference	Reference	Reference
Q2	1178(30.0)	331(15.7)		2.62 (2.13–3.24)	2.87 (2.32–3.55)	2.89 (2.33–3.57)
Q3	925(23.6)	584(27.7)		5.89 (4.83–7.20)	6.64 (5.42–8.15)	6.67 (5.43–8.18)
Q4	458(11.7)	1051(49.8)		21.42 (17.49–26.24)	24.44 (19.84–30.10)	24.47 (19.85–30.16)
HOMA-beta	113.04±176.90	135.70±142.38	<0.001	1.21 (1.12–1.31)	1.31 (1.20–1.43)	1.30 (1.19–1.42)
Q1	1072(27.3)	437(20.7)	<0.001	Reference	Reference	Reference
Q2	1092(27.8)	417(19.7)		0.94 (0.80–1.10)	1.02 (0.87–1.20)	1.02 (0.87–1.20)
Q3	964(24.6)	545(25.8)		1.39 (1.19–1.62)	1.61 (1.37–1.88)	1.60 (1.37–1.88)
Q4	796(20.3)	713(33.8)		2.20 (1.89–2.55)	2.79 (2.38–3.27)	2.79 (2.38–3.28)
Indicators on inflammation						
CRP	2.00±4.36	2.44±3.95	<0.001	1.10 (1.05–1.16)	1.10 (1.04–1.15)	1.09 (1.04–1.15)
Q1	1172(29.9)	355(16.8)	<0.001	Reference	Reference	Reference
Q2	1063(27.1)	455(21.5)		1.41 (1.20–1.66)	1.39 (1.18–1.63)	1.39 (1.18–1.64)
Q3	887(22.6)	602(28.5)		2.24 (1.91–2.62)	2.18 (1.86–2.56)	2.19 (1.87–2.57)
Q4	802(20.4)	700(33.1)		2.88 (2.47–3.37)	2.81 (2.40–3.28)	2.81 (2.40–3.30)
WBC	5.73±1.48	6.33±1.55	<0.001	1.49 (1.41–1.57)	1.51 (1.43–1.60)	1.52 (1.44–1.61)
Q1	1196(30.5)	320(15.2)	<0.001	Reference	Reference	Reference
Q2	1030(26.2)	483(22.9)		1.75 (1.49–2.07)	1.76 (1.49–2.07)	1.76 (1.49–2.08)
Q3	893(22.8)	612(29.0)		2.56 (2.18–3.01)	2.61 (2.22–3.07)	2.62 (2.23–3.09)
Q4	805(20.5)	697(33.0)		3.24 (2.76–3.80)	3.35 (2.85–3.95)	3.43 (2.91–4.04)
LYMPH	1.99±0.59	2.24±0.61	<0.001	1.51 (1.43–1.59)	1.50 (1.42–1.58)	1.51 (1.43–1.60)
Q1	1194(30.4)	336(15.9)	<0.001	Reference	Reference	Reference
Q2	1037(26.4)	480(22.7)		1.65 (1.40–1.94)	1.64 (1.40–1.93)	1.66 (1.41–1.96)
Q3	918(23.4)	564(26.7)		2.18 (1.86–2.56)	2.17 (1.85–2.55)	2.19 (1.86–2.57)
Q4	775(19.8)	732(34.7)		3.36 (2.87–3.93)	3.30 (2.81–3.86)	3.36 (2.86–3.94)

Notes: Continuous variables are described as mean ± SD, categorical variables are shown as number (percentage). For continuous variables, the unit for OR estimate is SD (calculated from the whole population). Model 1, univariable model; Model 2, adjusted for sex, age (35–44, 45–54, 55–64, 65–75 years); Model 3, further adjusted for educational level (0, 1–6, 7–9, >9 years), current alcohol drinking (yes, no), current smoking (yes, no), physical activity (low, moderate, high) in addition to those included in model 2.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; IR, insulin resistance; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-beta, homeostatic model assessment of beta-cell function index; CRP, C reactive protein; WBC, white blood cell count; LYMPH, lymphocyte count.

HOMA-IR, HOMA-beta, CRP, WBC, and LYMPH. Among these, the standardized β of all defined obesity for insulin (0.28–0.35) and HOMA-IR (0.23–0.30) were higher than those for others (Table S3).

The Mediation Role of IR and Inflammation in the Association Between Obesity and NAFLD in the Fuqing Cohort Study

The previous results indicate that various types of obesity are significantly associated with an increased risk of NAFLD. Individuals with obesity exhibit higher levels of IR and inflammation compared to those without obesity. Moreover, both IR and inflammatory indicators are positively correlated with the risk of NAFLD. Building on these findings, we performed a mediation analysis to evaluate the indirect effect of IR and inflammation in the association of each type of obesity with NAFLD risk.

Table 3 shows the results of mediation analyses. All indirect relationship estimates were statistically significant. Compared with other indicators, insulin and HOMA-IR were more strongly associated with the link between obesity and NAFLD, with

Table 3 Mediation Analysis With Insulin Resistance and Inflammation Indicators as Potential Mediators Between Obesity and NAFLD Risk in Fuqing Cohort Study

Exposure	Mediator	NAFLD, OR (95% CI) *		Overall Proportion Mediated
		Direct Effect	Indirect Effect	% (95% CI)
Obesity_BMI				
	Insulin	3.86 (3.23–4.56)	2.21 (2.02–2.42)	61.92 (57.99–66.00)
	HOMA-IR	3.57 (2.97–4.15)	2.44 (2.18–2.61)	66.72 (61.91–69.40)
	HOMA-beta	7.30 (6.09–8.72)	1.17 (1.12–1.22)	16.27 (12.30–20.00)
	CRP	7.57 (6.20–8.98)	1.12 (1.09–1.17)	12.39 (9.51–16.60)
	WBC	7.67 (6.30–8.92)	1.11 (1.07–1.16)	11.23 (7.71–15.50)
	LYMPH	7.56 (6.24–8.83)	1.13 (1.09–1.18)	13.13 (9.59–17.00)
	Total	3.66 (2.97–4.37)	2.72 (2.44–3.03)	70.30 (66.59–73.70)
Obesity_WC				
	Insulin	3.50 (3.19–3.96)	1.91 (1.78–2.04)	56.03 (52.22–59.50)
	HOMA-IR	3.22 (2.97–3.67)	2.03 (1.95–2.21)	59.80 (57.54–64.40)
	HOMA-beta	6.01 (5.45–6.84)	1.12 (1.08–1.15)	12.45 (9.07–15.10)
	CRP	6.08 (5.51–6.90)	1.11 (1.07–1.14)	11.18 (8.02–14.10)
	WBC	5.96 (5.39–6.64)	1.13 (1.10–1.16)	13.61 (10.30–16.50)
	LYMPH	6.01 (5.43–6.69)	1.11 (1.08–1.15)	11.97 (9.20–15.20)
	Total	3.19 (2.87–3.55)	2.35 (2.18–2.55)	66.37 (62.61–69.70)
Obesity_WHR				
	Insulin	3.21 (2.79–3.72)	1.72 (1.63–1.83)	50.97 (47.70–55.30)
	HOMA-IR	2.93 (2.56–3.42)	1.88 (1.77–2.01)	57.29 (53.59–61.40)
	HOMA-beta	5.01 (4.35–5.73)	1.11 (1.08–1.14)	12.10 (8.97–14.30)
	CRP	5.05 (4.40–5.75)	1.09 (1.07–1.13)	10.58 (8.20–14.10)
	WBC	4.91 (4.22–5.65)	1.13 (1.10–1.16)	13.76 (10.87–17.00)
	LYMPH	4.98 (4.31–5.67)	1.11 (1.08–1.15)	11.98 (9.06–15.50)
	Total	2.93 (2.54–3.42)	2.15 (1.98–2.29)	63.54 (59.09–67.10)
Obesity_WHtR				
	Insulin	4.95 (4.16–5.71)	1.90 (1.77–2.03)	53.12 (48.79–56.50)
	HOMA-IR	4.57 (3.90–5.31)	2.06 (1.91–2.20)	57.46 (54.03–60.80)
	HOMA-beta	8.39 (7.14–9.67)	1.10 (1.08–1.15)	10.47 (8.17–14.40)
	CRP	8.48 (7.26–9.83)	1.10 (1.08–1.13)	10.54 (7.95–12.90)
	WBC	8.16 (6.96–9.46)	1.13 (1.11–1.17)	12.97 (11.11–16.60)
	LYMPH	8.25 (7.08–9.64)	1.13 (1.10–1.16)	12.81 (9.92–15.40)
	Total	4.46 (3.74–5.10)	2.36 (2.15–2.54)	63.60 (59.62–67.00)

Notes: All mediators were categorized into quartiles. * Adjusted for sex, age (35–44, 45–54, 55–64, 65–75 years), educational level (0, 1–6, 7–9, >9 years), current alcohol drinking (yes, no), current smoking (yes, no), and physical activity (low, moderate, high).

Abbreviations: NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-beta, homeostatic model assessment of beta-cell function index; CRP, C reactive protein; WBC, white blood cell count; LYMPH, lymphocyte count; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

HOMA-IR emerging as the strongest mediator for all exposures. The indirect effect of insulin and HOMA-IR accounted for a non-negligible proportion (50.97–61.92% for insulin, 57.29–66.72% for HOMA-IR) of the associations between all types of obesity and NAFLD risk, while the proportion of HOMA-beta, CRP, WBC, LYMPH were all less than 17% (Table 3).

Sensitivity Analysis

Using the substitution of IR and inflammation with TyG and SIRI, the significant positive associations with NAFLD risk were retained, and the ORs of TyG were higher than those of SIRI (Table S4). The results of the mediation analyses shown in Table S5 also suggested that the TyG played a more important role in the association between obesity and NAFLD compared to that of SIRI. After further adjusting diabetes status, the results remained highly consistent with those reported above (Tables S6 and S7).

NHANES

The Demographic Characteristics of Participants in NHANES

A total of 5167 individuals were finally included in our study, of which 56.0% were female, with an average age of (53.19±11.01) years (Table S8). The prevalence of NAFLD in the total population was 41.61%. Consistent with the results of the Fuqing Cohort Study described above, NAFLD patients had a higher prevalence of obesity and central obesity, compared to non-cases.

Associations of Obesity, IR, Inflammation, and NAFLD in NHANES

Table S9 presents the results of the multivariable logistic regression analysis. Following multivariable adjustments, it was observed that obesity, indicators of IR, and indicators of inflammation were all significantly associated with an increased risk of NAFLD. These findings corroborate previous results from the Fuqing cohort study. Compared to the Fuqing cohort, the associations between obesity, IR, and inflammation with NAFLD were more pronounced in the NHANES population, with higher ORs observed.

Mediation Analysis in NHANES

In our mediation analysis based on the NHANES population, we found results similar to those from the Fuqing cohort study, demonstrating a significant mediating effect of IR and inflammation in the association between obesity and NAFLD (Table 4). Specifically, the mediation proportions provided by IR indicators, such as HOMA-IR, were

Table 4 Mediation Analysis With Insulin Resistance and Inflammation Indicators as Potential Mediators Between Obesity and NAFLD Risk in NHANES 2007–2018

Exposure	Mediator	NAFLD, OR (95% CI) *		Overall proportion mediated
		Direct effect	Indirect effect	% (95% CI)
Obesity_BMI				
	Insulin	2.11 (1.96–2.38)	3.54 (3.28–4.01)	82.83 (80.21–85.20)
	HOMA-IR	2.02 (1.85–2.21)	3.90 (3.53–4.40)	85.17 (82.61–87.40)
	HOMA-beta	4.67 (4.12–5.24)	1.71 (1.58–1.76)	47.32 (42.48–49.50)
	CRP	5.91 (5.27–6.64)	1.30 (1.23–1.37)	26.37 (21.97–30.90)
	WBC	6.66 (5.97–7.61)	1.17 (1.11–1.20)	16.65 (11.53–18.80)
	LYMPH	7.20 (6.46–8.24)	1.07 (1.04–1.08)	7.06 (4.63–8.70)
	Total	2.03 (1.85–2.28)	4.97 (4.52–5.76)	88.68 (86.65–90.60)
Obesity_WC				
	Insulin	2.35 (2.07–2.61)	3.67 (3.28–4.03)	82.28 (79.49–84.50)
	HOMA-IR	2.24 (2.00–2.48)	4.01 (3.53–4.35)	84.48 (81.81–86.40)
	HOMA-beta	4.85 (4.32–5.73)	1.68 (1.58–1.80)	46.15 (42.23–50.30)
	CRP	6.29 (5.41–7.33)	1.34 (1.28–1.42)	28.86 (24.89–33.80)
	WBC	7.13 (6.19–8.46)	1.18 (1.13–1.21)	16.94 (12.64–19.40)
	LYMPH	7.74 (6.80–9.13)	1.07 (1.05–1.10)	7.31 (5.48–9.90)
	Total	2.27 (2.01–2.54)	5.34 (4.70–5.95)	88.57 (86.29–90.20)

(*Continued*)

Table 4 (Continued).

Exposure	Mediator	NAFLD, OR (95% CI) *		Overall proportion mediated
		Direct effect	Indirect effect	% (95% CI)
Obesity_WHtR				
	Insulin	4.03 (2.67–7.59)	5.94 (5.03–7.31)	86.80 (84.31–90.00)
	HOMA-IR	3.88 (2.47–7.68)	6.80 (5.21–8.62)	88.67 (85.17–91.30)
	HOMA-beta	13.09 (7.85–30.90)	2.02 (1.89–2.22)	52.38 (48.78–57.20)
	CRP	19.15 (11.00–47.45)	1.55 (1.46–1.66)	36.73 (32.62–41.60)
	WBC	22.96 (13.60–59.88)	1.23 (1.18–1.30)	19.72 (15.94–23.90)
	LYMPH	25.50 (15.20–66.41)	1.13 (1.09–1.17)	11.56 (8.89–14.90)
	Total	5.20 (2.77–14.97)	10.71 (8.04–13.79)	92.32 (89.50–94.40)

Notes: All mediators were categorized into quartiles. * Adjusted for sex, age, race, educational level, current alcohol drinking, current smoking, and physical activity.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NHANES, national health and nutrition examination survey; OR, odds ratio; CI, confidence interval; BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-beta, homeostatic model assessment of beta-cell function index; CRP, C reactive protein; WBC, white blood cell count; LYMPH, lymphocyte count; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

higher than those of inflammation indicators in the association of all obesity types with NAFLD. Overall, the results from both cohorts were consistent, with the total mediation proportions of IR and inflammation in the NHANES population being slightly higher than those observed in the Fuqing cohort (NHANES: 88.57%-92.32%, Fuqing cohort: 63.54–70.30%).

MR Analysis

All SNPs selected for inclusion in MR analysis are presented in Table S10. The IVW results showed that genetically predicted each SD higher BMI (OR: 2.05, 95% CI: 1.17–3.58), WC (OR: 2.26, 95% CI: 1.14–4.47), and WHR (OR: 1.90, 95% CI: 1.02–3.51) were all significantly associated with an increased risk of NAFLD (Table S11). Genetic IVs of all exposures showed no pleiotropy with those of NAFLD (Table S12), and possible heterogeneity was observed only for BMI (Table S13).

In the first step of the 2-step MR, all obesity-associated traits (including BMI, WC, and WHR) showed significant positive associations with most IR-related and inflammation-related indicators, except that no significant causal association was found between WHR and CRP (Table S14). Although a possible pleiotropy was observed in the association between WC and CRP ($P_{\text{pleiotropy}}$=0.018, Table S12), the relationship remained stable in the MR-Egger regression results, with a significant positive slope coefficient of 0.52 (95% CI: 0.33–0.70). In the second step, we found causal evidence for the effects of HOMA-IR (OR: 2.34, 95% CI: 1.14–4.78) and CRP (OR: 1.34, 95% CI: 1.04–1.73) on NAFLD (Table S15). No evidence of reverse causality was found across the analyses in the MR Steiger test (Table S16).

Finally, we estimated the indirect effect of obesity-related indicators on NAFLD. In the relationship between general obesity and NAFLD, only the indirect effects of HOMA-IR and CRP were statistically significant. HOMA-IR explained 21.38% of the total effect of BMI on NAFLD, and CRP explained 12.36% (Figure 2A). In the causal relationship between central obesity (defined by WC) and NAFLD (Figure 2B), the mediating effect explained by IR was similarly higher than that of inflammation (25.13% for HOMA-IR, 10.73% for CRP). For the WHR-NAFLD relationship, the statistically significant mediation effect was only found in HOMA-IR with a mediated proportion of 21.82% (Figure S4).

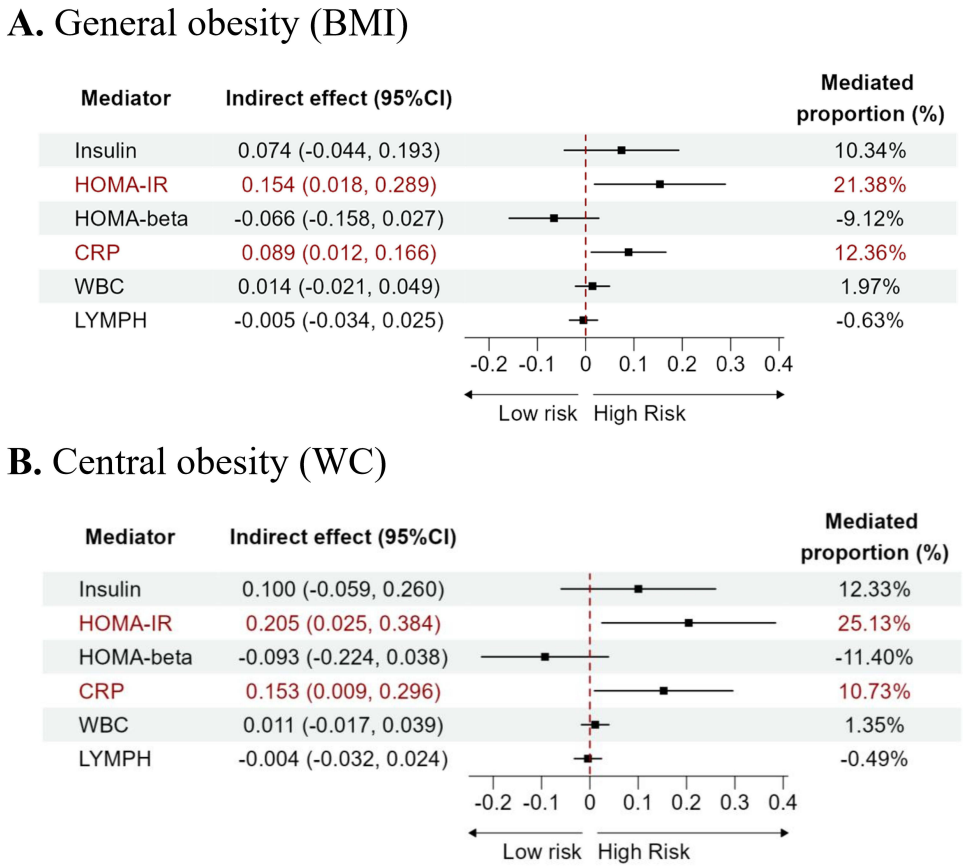

Figure 2 MR-estimated indirect effects of obesity phenotypes on NAFLD. (**A**) General obesity (defined by BMI); (**B**) Central obesity (defined by WC).
Abbreviations: MR, Mendelian randomization; NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; WC, waist circumference; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-beta, homeostatic model assessment of beta-cell function index; CRP, C reactive protein; WBC, white blood cell count; LYMPH, lymphocyte count; CI, confidence interval.

Discussion

In the current study, we explored the mediation effect of indicators on IR and chronic inflammation in the association between obesity and NAFLD in two cross-sectional studies and validated in MR analysis. Results from two cross-sectional studies indicated that all types of obesity are significantly associated with an increased risk of NAFLD. The total mediation effect of IR and chronic inflammation contributed more than 60% in the associations between all obesity phenotypes and NAFLD, and IR contributed more than chronic inflammation. MR analysis supported that obesity had a positive causal association with NAFLD. Additionally, IR and inflammation in individuals with obesity are significant contributors to the development of NAFLD, and the mediation proportion of IR was higher than chronic inflammation.

Excess body weight is closely associated with the increased risk of metabolic morbidity and mortality, triggered by the accumulation of adipose tissue in the human body. IR and systemic chronic inflammation are common in the context of obesity and are established instigators of various metabolic disorders.[45] NAFLD has been thought as a complex interplay of hepatic lipid accumulation,[46] and IR and inflammation have been widely used in NAFLD risk assessment. However, relatively little is known about their total and respective effects on obesity and NAFLD. In the current study, different chronic inflammation assessments, including CRP, WBC, and LYMPH according to our previous study,[6] were adopted to give a more comprehensive description of their effects. The results showed that the combined proportion mediated by insulin, HOMA-IR, CRP, WBC, and LYMPH ranged from 63.54% to 92.32% in the association between differently defined obesity and NAFLD. Interestingly, when looking at their respective effects, the mediation proportion of insulin and HOMA-IR were both higher than 50%, while the mediation proportions of chronic inflammation indicators

were 7.06%-36.73%, indicating a higher mediation effect of insulin and IR than chronic inflammation. To verify the results, the alternatives of IR and chronic inflammation were adopted in sensitivity analyses. TyG was a recently reported surrogate measure of IR,[47] and SIRI was developed as a comprehensive biomarker that could reflect the body's systemic inflammation.[48] The mediation proportion provided by TyG is still higher than SIRI.

Given that many unidentified factors may exist in the association of obesity and NAFLD, we adopted genetic variants to build a model and probe the causal conclusion using MR analysis.[20] The causal association of BMI, WC, and WHR to NAFLD were all significant, which was similar to the above-mentioned in the cross-sectional studies, and previous genetic evidence.[8–11] Intriguingly, the mediation proportions of HOMA-IR were all higher than 21%, while the proportions of CRP were < 13%. Although not as high as the gap in the above cross-sectional studies, the current MR analysis conveyed the information that HOM-IR may contribute more than CRP between obesity and NAFLD. Understanding the roles of IR and chronic inflammation in the association between obesity and NAFLD is crucial for targeted NAFLD monitoring and further mechanistic studies. The observed mediation proportions highlight that IR indicators, such as HOMA-IR, may be more significant than chronic inflammation markers (eg, CRP) in NAFLD monitoring, suggesting that monitoring IR levels in obese populations may aid in NAFLD prevention. Future research should include more comprehensive longitudinal studies to validate these findings and elucidate the roles of IR and chronic inflammation in the pathogenesis of NAFLD.

The primary strength of this study lies in its integration of a cross-sectional, general population-based design with MR analysis. MR analysis could avoid measurement errors, uncontrolled confounding, and reverse causality inherent in conventional epidemiological studies. However, our current results should be interpreted with causation due to limitations. First, only easily accessible indicators on IR were analyzed given that their gold standard methods were high-cost or complex, and could hardly apply to disease monitor or evaluation in public health practice. Second, the gold standard method of NAFLD diagnosis is liver biopsy, which is invasive and less acceptable in large-scale population studies. Thus, ultrasonography and USFLI were applied in the NAFLD diagnosis, which may introduce misclassification bias. Additionally, different diagnostic criteria were applied across various populations, potentially leading to further variability. Third, although we adjusted for known confounding factors, residual confounding may still exist due to the inherent limitations of observational studies. Fourth, in addressing the limited number of SNPs meeting the genome-wide significance threshold ($P<5\times10^{-8}$), we relaxed the SNP selection criteria. This approach may increase the risk of false positives. Furthermore, the constant presence of heterogeneity among SNPs, despite the use of a random-effects model, may introduce potential bias and affect the robustness of our MR results. Finally, our cross-sectional studies were from southeast China and the US, while genetic data in MR analysis consisted of European subjects. There may have unmeasured confounding factors for population heterogeneity, thus further large-scale population studies with multi-racial genetic backgrounds are necessary.

Conclusion

In summary, our present study provided both epidemiological and MR data in support of the causal roles of obesity traits (BMI, WC, and WHR) in NAFLD, and the mediation roles of IR (HOMA-IR) and inflammation (CRP) in their associations. We highlighted the vital role of HOMA-IR in obesity-associated NAFLD monitoring. Further confirmation in well-powered studies is warranted.

Abbreviations

BMI, body mass index; CI, confidence interval; CRP, C reactive protein; GIANT, genetic investigation of anthropometric traits; GWAS, genome-wide association studies; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA-beta, homeostatic model assessment of beta-cell function index; HOMA-IR, homeostatic model assessment of insulin resistance; IR, insulin resistance; IVs, instrumental variables; IVW, inverse variance weighted; LD, linkage disequilibrium; LYMPH, lymphocyte count; MAGIC, meta-analyses of glucose and insulin-related traits consortium; MET, metabolic equivalent; MR, Mendelian randomization; NAFLD, nonalcoholic fatty liver disease; NHANES, national health and nutrition examination survey; OR, odds ratio; SD, standardized deviation; SIRI, systemic inflammation response index; SNPs,

single nucleotide polymorphisms; TyG: triglyceride-glucose index; USFLI, United States fatty liver index; WBC, white blood cell count; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding authors upon reasonable request.

Ethics Approval and Consent to Participate

The Ethics Review Committee of Fujian Medical University approved the study protocol (approval number, [2017-07] and [2020-58]) before data collection, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all participants.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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