Associations of Obesity-metabolic Status With Insulin Resistance and Chronic Inflammation Level: Results From the CNTR Study

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Research

Keywords: Metabolic status, Insulin resistance, Chronic inflammation, Twin study

Posted Date: December 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-132409/v1

Abstract

Background: Insulin resistance (IR) and inflammation are the potential mechanism linking obesity and cardiometabolic risk. The aim of this work was to examine the joint relations of obesity and metabolic status with IR and chronic inflammation level among Chinese adult twins.

Methods: The analyses used data from 1113 adult twins in 4 provinces (Shandong, Zhejiang, Jiangsu and Sichuan) from Chinese National Twin Registry (CNTR) which collected detailed information. Those with 0 or 1 metabolic syndrome (MetS) components excluding waist circumference were considered metabolically healthy, and those with waist circumference≥90 cm in men and ≥85 cm in women as obese. All participants were categorized into four phenotypes: metabolically healthy non-obesity (MHNO), metabolically healthy obesity (MHO), metabolically unhealthy non-obesity (MUNO), metabolically unhealthy obesity (MUO). High sensitivity C reactive protein (hsCRP) was measured to assess underlying inflammation and homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as surrogate measure of insulin resistance.

Results: In observational analyses of 1113 individuals (mean [SD] age, 46.6 [12.9] years; 463 obese [41.6%]). 20.3% obese twins were metabolic healthy. Serum HOMA-IR level was higher in MUNO (β=0.42, 95% CI: 0.21–0.64), MHO (β=0.68, 95% CI: 0.36–1.00) and MUO (β=0.69, 95% CI: 0.46–0.91) twins, compared with their MHNO counterparts. The chronic inflammation level, evaluated by hsCRP was similar between MHO and MUO, which differed significantly to metabolic healthy non-obesity (MHNO). Within twin-pair analysis indicated there might exist common genetic influence between HOMA-IR and MHO/MUO phenotype.

Conclusions: Among Chinese adult twins, metabolic status were independently associated with higher IR while development of chronic inflammation might closely relate to central obesity. It is necessary for the different risk assessment based on metabolic status in obese population.

Introduction

Obesity is often associated with a constellation of metabolic abnormalities, including insulin resistance, impaired fasting glucose and/or tolerance, dyslipidemia, hypertension and metabolic syndrome (MetS), which are important risk factors for type 2 diabetes (T2D) and cardiovascular diseases (CVDs)(1, 2). However, not all obese individuals entail metabolic abnormalities. The healthier phenotype displayed by some obese patients has been called metabolically healthy obesity (MHO). In general, the risks of T2D, CVDs, and all-cause mortality in those with MHO are lower than people with metabolic unhealthy obesity (MUO), but greater than in those who are metabolically healthy and non-obese (MHNO)(3–7), although inconsistent results have also been reported (8, 9). Moreover, recent studies have found that the risks of these adverse outcomes are directly related to the number and severity of metabolic abnormalities (10–13).

It is now generally accepted insulin resistance and inflammation are the potential mechanism linking obesity and cardiometabolic risk (14, 15). Insulin resistance is identified as an impaired biologic response to insulin stimulation of target tissues, primarily the liver, muscle, and adipose tissue. The metabolic consequences of insulin resistance can result in hyperglycemia, hypertension, dyslipidemia, visceral adiposity and contributing to cardiometabolic syndrome and increased CVD risk (16). Studies have found that insulin sensitivity is greater in people with MHO than in those with MUO, and many participants identified as having MHO are more insulin resistant than those who are MHNO(17–19).

Experimental and observational evidence also suggested that inflammation might play a central role in the pathogenesis of cardiovascular disease. Some studies reported that MHO participants had significantly lower CRP levels than non-MHO (20, 21). Karelis et al. reported that MHO women had a lower inflammatory state than women with postmenopausal insulin resistance and that this had a role in lowering the risk of cardiovascular disease (20). There are also studies found a similar level of CRP between two groups (22, 23). To date inconsistent findings were reported about the relationship between inflammation level and MHO/MUO phenotype.

The factors responsible for the greater preservation of insulin action in people with MHO than in those with MUO are not clear, but could be related to differences in potentially modifiable lifestyle factors and genetic factors associated with adipose tissue biology. MHO is more often observed in young, physically active patients with a better nutritional status and low levels of ectopic and visceral fat storage (24). GWAS have identified genetic variants that are associated with increased adiposity in conjunction with a healthy metabolic profile (25). As most previous studies were unable to control for the individual genetic variability it was unknown whether associations between metabolic abnormalities and clinical characteristics were attributable to shared genetic vulnerabilities influencing both phenotypes. Twin design is seen as a useful method of controlling confounders in observational epidemiologic studies. Especially monozygotic (MZ) twins who are completely matched for any variations in the genetic background provide an extremely powerful control for genetic confounding factors.

Therefore, it is necessary to clarify the insulin resistance and chronic inflammation level of obese people that have significant metabolic abnormalities controlling genetic and familial factors. In this study, we aimed to investigate the joint relations of obesity and metabolic status with insulin resistance and inflammation in a large cohort of Chinese twin adults.

Methods

Study Population

The participants belong to the Chinese National Twin Registry (CNTR), the first and largest population-based twin registry in China described in detail elsewhere (26).

The analyses in this paper were based on a follow-up survey held from April to December 2013 among 1147 participants. The subjects were adult twins from four provinces covering 9 cities in Shandong, Zhejiang, Jiangsu and Sichuan province who completed an in-person questionnaire interview, a physical examination and a fasting blood biochemical test.

Pregnant female twins were excluded from participation. Twins were excluded from analyses if: (1) with a definitive diagnosis of medical diseases such as alimentary tract tumor, cardiovascular heart disease, stroke and kidney disease; (2) treated with weight-lowering pharmacological agents. At last, a total of 1113 individuals (541 completed twin pairs and 31 individuals) were eligible for this study.

Determination of zygosity was based on the information from questionnaires during the baseline investigation. Twins of different genders were directly classified as DZ. For twins of the same gender, a model was built according to age, gender and 'whether they were as alike as two peas in a pod'. The model has been validated using DNA genotyping and found to be >90% accurate (27). All participants provided their written informed consent and Biomedical Ethics Committee at Peking University, Beijing, China approved the study protocol.

Clinical and Biochemical Data Collection

Data were collected with standardized computer-assisted personal interviews and medical examinations by trained staff. Information on demographic characteristics, medical history, and lifestyle factors were recorded, including questions on tobacco smoking (never, former, current), alcohol drinking (never, former, current) and exercise activities. Participants' exercise activities on occupation, transportation, daily life and leisure time were assigned a metabolic equivalent task (MET) value, using the Compendium of Physical Activities by Ainsworth et al.(28).

In addition, each participant's blood pressure, height, weight and percent body fat (PBF) were measured. Blood pressure was calculated as the mean of the second and third measurement out of three consecutive measurements. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Height was measured to the nearest 0.1 cm on a portable stadiometer while weight was measured to the nearest 0.1 kilograms using a digital balance (Body Composition Analyzer/Scale, TANITA, Tokyo, Japan). Waist circumference was measured three times at the level of the umbilicus to the nearest centimeter and the mean value was used in the analyses. PBF was determined by bioelectrical impedance (Body Composition Analyzer/Scale, TANITA).

Venous blood samples were collected, and serum total cholesterol (TC) and triglycerides (TG) were measured by the enzymatic colorimetric method (Roche, Basel, Switzerland). Direct methods were applied to assess high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol (Roche, Basel, Switzerland). A modified hexokinase enzymatic method was used to detect glucose (Glu) (Roche, Basel, Switzerland), and serum insulin was measured by chemiluminescence immunoassay (CLIA) on the ADVIA Centaur immunoassay system. Insulin resistance was estimated according to homeostasis model assessment (HOMA-IR): HOMA-IR = [fasting glucose (mmol/l) × insulin (U/ml)]/22.5. Serum high-sensitivity CRP (hsCRP) was measured using a high-sensitivity immunoturbidimetric method (CRP [Latex] HS, Roche, Mannheim, Germany) on a Hitachi auto-analyzer (Roche Diagnostics, Mannheim, Germany). To minimize the effects of assay variability, samples from each twin pair were analyzed using the same assay.

Definition of the Phenotypes

We considered four components of the metabolic syndrome: 1) systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or self-reported hypertension or using antihypertensive drugs; 2) serum fasting glucose ≥5.6 mmol/L or self-reported diabetes or intake of antidiabetic medication; 3) HDL cholesterol <1.0 mmol/L for men and <1.3 mmol/L for women or using lipid-lowing drugs; and 4) triglycerides ≥1.7 mmol/L or using lipid-lowing drugs.. Individuals with waist circumference ≥90 cm (men) and 85 cm (women) were considered obese(29). According to the NCEP-ATP III criteria(30), participants with ≤1 abnormal component excluding waist circumference were defined as metabolically healthy (MH), with the remaining defined as metabolically unhealthy (MU). Status of metabolic health and obesity categories were combined to create the four phenotypes: metabolically healthy non-obesity (MHNO), metabolically healthy obesity (MHO), metabolically unhealthy non- obesity (MUNO), metabolically unhealthy obesity (MUO).

Statistical Methods

Data are presented as mean ± SD or median (first quartile (Q1) – third quartile (Q3)) for continuous variables and absolute and relative frequencies for categorical variables. We compared epidemiological, physical and biochemical characteristics between MHO and MUO twins as well as MHNO and MUNO twins. P values were corrected for the correlation between co-twins using generalized estimating equations.

Mixed-effect linear regression models with a random intercept for each twin pair to account for twin clustering were performed to examine the relationship of serum HOMA-IR and hsCRP levels with metabolic status (MH as reference group), number of MetS components, and the combined obesity-metabolic categories (MHNO as reference group),with adjustment for potential covariates. The first model was adjusted for age, sex, place, and zygosity; the second model was additionally adjusted for lifestyle factors (smoking, drinking, and MET level), obesity indicators (BMI, PBF, WHR) were further adjusted in the final model.

To investigate whether these associations were confounded by shared genetic and environmental factors, we applied co-twin regression analyses within twin pairs stratified by zygosity. The within-pair approach automatically takes into account shared familial and environmental influences. Fixed effect models were used to estimate the relation of serum HOMA-IR and hsCRP levels with metabolic status (MH as reference group), number of MetS components and the combined obesity-metabolic categories (MHNO as reference group) separately for DZ and MZ twins adjusted for lifestyle factors (smoking, drinking, and physical activity) and obesity indicators (BMI, PBF, WHR).

All the serum metabolites were handled after logarithmic transformation in the regression analyses. Robust standard error and confidence intervals for estimates have been produced. All the statistical analyses were performed with Stata statistical software (release 12.0; Stata Corporation, College Station, TX). P-values are two-sided, and statistical significance was assumed at $P<0.05$.

Results

Sample characteristics

This analysis included 1113 participants (46.55 ± 12.89 years, 65.4% men,61.8% MZ) of whom 41.6% were obese. Ninety four participants (8.4%) were classified in the MHO group, 369 (33.2%) in the MUO group, 233 (20.9%) in the MHNO group, and 417 (37.5%) in the MUNO group (Table 1). The MHO individuals had a mean age of 40.85 ± 12.03 years with a high proportion of women (52.1%). When this group was compared to the MUO phenotype, differences were found in lower WC, WHR ($p < 0.0001$) and lower concentrations of most biochemical characteristics except hsCRP. Besides, the MHO group had the lowest proportion of current smokers (18.1%) and current drinkers (16.0%) and highest reported MET levels among four groups. Compared to MUNO, MHNO individuals were younger, with a higher proportion of women (48.9%), and were less likely to smoke and drink.

Table 1
Baseline characteristics of the study participants (N = 1113).

Characteristic	All (*n* = 1113)	Obesity(n = 463)		*p* *	Non-obesity(n = 650)		*p* *
		MHO (n = 94)	MUO (n = 369)		MHNO (n = 233)	MUNO (n = 417)	
Age,mean(SD;years)	46.55(12.89)	40.85(12.03)	47.07(11.60)	< 0.001	40.20(12.80)	50.92(12.31)	< 0.001
Sex, n (%)							
Male	728(65.4%)	45(47.9%)	277(75.1%)		119(51.1%)	287(68.8%)	
Female	385(34.6%)	49(52.1%)	92(24.9%)	< 0.001	114(48.9%)	130(31.2%)	< 0.001
zygosity, n (%)							
MZ	688(61.8%)	49(52.1%)	228(61.8%)		134(57.5%)	277(66.4%)	
DZ	425(38.2%)	45(47.9%)	186(38.2%)	0.113	99(42.5%)	140(33.6%)	0.030
Place,n (%)							
Qingdao	218(19.6%)	16(17.0%)	96(26.0%)		29(12.5%)	77(18.5%)	
Jiangsu	437(39.2%)	37(39.4%)	133(36.0%)	0.203	94(40.3%)	173(41.5%)	0.211
Sichuan	122(11.0%)	12(12.8%)	94(40.3%)	0.145	36(15.5%)	37(8.9%)	0.009
Zhejiang	336(30.2%)	29(30.9%)	173(41.5%)	0.196	74(31.8%)	130(31.2%)	0.147
Smoking, n (%)							
Never smoker	621(56.2%)	71(75.5%)	170(46.6%)		160(69.3%)	220(53.1%)	
Current smoker	362(32.8%)	17(18.1%)	146(40.0%)	< 0.001	56(24.2%)	143(34.5%)	0.016
Former smoker	121(11.0%)	6(6.4%)	49(13.4%)	0.005	15(6.5%)	51(12.3%)	0.003
Drinking, n (%)							
Never drinker	715(64.8%)	77(81.9%)	222(61.2%)		177(76.6%)	239(57.5%)	
Current drinker	365(33.1%)	15(16.0%)	132(36.4%)	0.002	50(21.7%)	168(40.4%)	< 0.001
Former drinker	24(2.2%)	2(2.1%)	9(2.5%)	0.443	4(1.7%)	9(2.2%)	0.932
Physical acitivities,mean(SD;MET/week)	5986.2(6797.2)	6634.7(6961.8)	5649.8(6565.7)	0.564	6335.5(6605.9)	5928.3(7063.8)	0.526
BMI,mean(SD;kg/m^2)	24.48(3.62)	27.10(2.81)	27.44(2.83)	0.071	21.58(2.52)	22.92(2.47)	< 0.001
WC,mean(SD;cm)	86.11(10.45)	93.00(5.94)	96.02(6.91)	0.001	76.82(6.86)	80.95(6.53)	< 0.001
WHR,mean(SD)	0.90(0.09)	0.92(0.05)	0.95(0.13)	< 0.001	0.84(0.05)	0.87(0.05)	< 0.001
PBF,mean(SD)	27.45(11.34)	33.33(9.88)	31.50(11.75)	0.161	23.64(8.76)	24.69(10.90)	0.149
SBP,median (IQR; mmHg)	131.50(25.50)	121.50(14.00)	139.00(22.00)	< 0.001	115.50(15.08)	136.50(23.83)	< 0.001
DBP ,median (IQR; mmHg)	79.33(15.00)	75.50(11.00)	85.00(13.67)	< 0.001	70.50(9.33)	81.00(13.50)	< 0.001
TG,median (IQR; mmol/L)	1.32(1.12)	1.23(0.52)	1.84(1.53)	< 0.001	0.94(0.52)	1.32(1.17)	< 0.001
TC ,median (IQR; mmol/L)	4.77(1.35)	4.31(1.04)	5.13(1.17)	< 0.001	4.20(1.06)	4.87(1.31)	< 0.001
HDL-C,median (IQR; mmol/L)	1.38(0.43)	1.26(0.45)	1.35(0.40)	0.004	1.35(0.45)	1.46(0.45)	0.001
LDL-C ,median (IQR; mmol/L)	2.15(0.77)	2.01(0.64)	2.37(0.73)	< 0.001	1.85(0.66)	2.19(0.78)	< 0.001

Characteristic	All (*n* = 1113)	Obesity(n = 463)		*p* *	Non-obesity(n = 650)		*p* *
		MHO (n = 94)	MUO (n = 369)		MHNO (n = 233)	MUNO (n = 417)	
Fasting glucose,median (IQR; mmol/L)	5.30(1.12)	4.97(0.78)	5.67(1.24)	< 0.001	4.91(0.63)	5.58(1.30)	< 0.001
HOMA-IR,median (IQR)	2.03(1.98)	2.26(1.67)	2.94(2.62)	0.022	1.34(1.30)	1.74(1.78)	< 0.001
Uric_acid,median (IQR; μmol/L)	327.25(124.95)	321.30(107.10)	368.90(124.95)	< 0.001	285.60(101.15)	327.25(119.00)	< 0.001
hsCRP,median (IQR; mg/L)	0.81(1.20)	0.99(1.47)	1.20(1.45)	0.774	0.45(0.70)	0.74(0.98)	0.164
Numerical data are presented as means (SD) or median (IQR), and categorical variables are presented as numbers (%).							
Abbreviations: MZ = monozygotic; DZ = dizygotic; MET = Metabolic Equivalent of Task; BMI = body mass index; WC = waist circumstance; WHR = waist hip ratio; PBF = percent body fat; SBP = systolic blood pressure; DBP = diastolic blood pressure; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol; HOMA-IR = homeostasis model assessment of insulin resistance; hsCRP = high-sensitivity CRP; IQR = interquartile range; SD = standard deviation; MHO = metabolically healthy obesity; MUO = metabolically unhealthy obesity; MHNO = metabolically healthy non-obesity; MUNO = metabolically unhealthy non-obesity;							

Metabolic status with insulin resistance, and chronic inflammation level

The associations of serum HOMA-IR and hsCRP levels with metabolic status are shown in Table 2. In the mixed linear models adjusted for sex, zygosity, place and age, MU phenotype was associated with a higher level of serum HOMA-IR(β = 0.52,95% CI: 0.43–0.62, p < 0.001) and hsCRP(β = 0.34,95% CI: 0.20–0.47, p < 0.001). After adjusted for lifestyle factors (smoking, drinking, and physical activity) and obesity indicators (model 3), the associations remained significant thought a slight decrease. We then repeated the analysis in obesity individuals. Compared with MHO, MUO group presented higher level of insulin resistance(β = 0.32,95% CI: 0.17–0.47, p < 0.001) but similar hsCRP level(β = 0.11,95% CI: -0.09–0.32, p = 0.277).

Table 2
Results of mixed linear regression models on the association of serum HOMA-IR and hsCRP levels with metabolic status in all twins

Exposure	Model 1		Model 2		Model 3	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
HOMA-IR						
Metabolic status						
MH(ref)						
MU	0.52(0.43,0.62)	< 0.001	0.53(0.43,0.62)	< 0.001	0.32(0.22,0.41)	< 0.001
Metabolic status in obesity						
MHO(ref)						
MUO	0.38(0.23,0.53)	< 0.001	0.40(0.24,0.56)	< 0.001	0.32(0.17,0.47)	< 0.001
CRP						
Metabolic status						
MH(ref)						
MU	0.34(0.20,0.47)	< 0.001	0.34(0.20,0.48)	< 0.001	0.17(0.03,0.30)	0.018
Metabolic status in obesity						
MHO(ref)						
MUO	0.20(-0.01,0.41)	0.057	0.18(-0.03,0.40)	0.09	0.11(-0.09,0.32)	0.277
Model 1 was adjusted for sex, zygosity, place and age; model 2 was adjusted for model 1 plus lifestyle factors (smoking, drinking and physical activity); model 3 was adjusted for model 2 plus BMI, PBF and WHR.						
Abbreviations: HOMA-IR = homeostasis model assessment of insulin resistance; hsCRP = high-sensitivity CRP; MH = metabolically healthy; MU = metabolically unhealthy; MHO = metabolically healthy obesity; MUO = metabolically unhealthy obesity; BMI = body mass index; PBF = percent body fat; WHR = waist hip ratio.						

Further, we analyzed the associations between MetS components and serum HOMA-IR and hsCRP levels. Individuals with 2 or more MetS components had a significantly higher level of HOMA-IR than normal individuals and a dose-response relation was found between numbers of MetS components and serum HOMA-IR after adjusting for obesity indicators and other covariates (P for trend < 0.001, Table S1). The association between numbers of MetS components and serum hsCRP began no more significant in the model additionally adjusted for obesity indicators. Stratified analysis according to obesity status indicated that serum hsCRP was related to number of MetS components only in obesity twins (Table S2 and S3).

Obesity-metabolic categories with insulin resistance, and chronic inflammation level

When combined metabolic status with obesity status, compared to MHNO individuals, the MUO group where both obesity and metabolic abnormalities were present, was characterized with the highest measurements for both indicators, followed by the MHO group. The MUNO group reflecting individuals with absence of obesity, but presence of metabolic abnormalities, was associated with the minimum increase in serum HOMA-IR and hsCRP levels. After adjusted for lifestyle factors (smoking, drinking, and physical activity), TC and HbA1c (model 3), all the associations were still significant thought there were a slight decrease (Table 3).

Table 3
Results of mixed linear regression models on the association of serum HOMA-IR and CRP levels with obesity-MetS categories in all twins

Exposure	Model 1		Model 2		Model 3	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
HOMA-IR						
Obesity–MetS phenotype						
MHNO (ref)						
MUNO	0.46(0.35,0.57)	< 0.001	0.48(0.37,0.59)	< 0.001	0.39(0.28,0.51)	< 0.001
MHO	0.54(0.39,0.69)	< 0.001	0.53(0.37,0.68)	< 0.001	0.51(0.36,0.67)	< 0.001
MUO	0.90(0.79,1.01)	< 0.001	0.90(0.78,1.01)	< 0.001	0.79(0.67,0.91)	< 0.001
hsCRP						
Obesity–MetS phenotype						
MHNO (ref)						
MUNO	0.30(0.14,0.46)	< 0.001	0.33(0.17,0.50)	< 0.001	0.27(0.10,0.44)	0.002
MHO	0.57(0.35,0.80)	< 0.001	0.60(0.37,0.82)	< 0.001	0.59(0.36,0.81)	< 0.001
MUO	0.72(0.55,0.88)	< 0.001	0.71(0.55,0.88)	< 0.001	0.63(0.46,0.80)	< 0.001
Model 1 was adjusted for sex, zygosity, place and age; model 2 was adjusted for model 1 plus lifestyle factors (smoking, drinking and physical activity); model 3 was adjusted for model 2 plus TC and HA1bc.						
Abbreviations: HOMA-IR = homeostasis model assessment of insulin resistance; hsCRP = high-sensitivity CRP; MetS = metabolic syndrome; MHO = metabolically healthy obesity; MUO = metabolically unhealthy obesity; MHNO = metabolically healthy non-obesity; MUNO = metabolically unhealthy non-obesity; TC = total cholesterol.						

We further classified metabolic health by the number of MetS components (0, 1, 2, 3, 4) and examined the joint relation of obesity groups and MetS numbers with serum HOMA-IR and hsCRP levels (Fig. 1). In multivariable-adjusted models, the levels of HOMA-IR were highest in individuals with obesity and 4 MetS components (β = 1.21,95% CI: 0.95–1.47, p < 0.001), followed by non-obesity individuals with 4 MetS components (β = 0.91,95% CI: 0.62–1.19, p < 0.001), and obese individuals with 3 MetS components (β = 0.88,95% CI: 0.65–1.12, p < 0.001) compared with those in non-obesity without any MetS component. With respect to levels of hsCRP, compared with those in non-obesity without any MetS component, participants with obesity and any MetS component had similar higher levels of hsCRP.

Within twin-pair analysis

In analyses controlling for genetic and familial effects within 541 complete twin pairs, associations of metabolic status with serum HOMA-IR and hsCRP levels are presented in Table 4. Compared to MH group, the MU group had significantly higher levels of HOMA-IR in both MZ and DZ twin-pair analysis adjusting for obesity indicators and other covariates. Besides, we analyzed the independent associations between MetS components with serum HOMA-IR and hsCRP levels. The results showed that compared with twins without any MetS component, level of serum HOMA-IR were

significantly higher in twins with 4 MetS components in both MZ and DZ twin-pair analysis. Among obesity group, when compared with obese twins without any MetS component, obese twins with 4 MetS components had significant higher level of serum HOMA-IR(β = 1.01,95% CI: 0.07–1.96, p = 0.036,S4 Table).

Table 4
Results of fixed linear regression models on the association of serum HOMA-IR and hsCRP levels with metabolic status in twin pairs

Exposure	MZ		DZ	
	β (95% CI)	p	β (95% CI)	p
HOMA-IR				
Metabolic status				
MH(reference group)				
MU	0.18(0.01,0.35)	0.037	0.28(0.08,0.48)	0.006
Metabolic status in obesity				
MHO(ref)				
MUO	-0.06(-0.46,0.34)	0.751	**0.72(0.24,1.20)**	**0.005**
CRP				
Metabolic status				
MH(reference group)				
MU	0.00(-0.31,0.32)	0.985	0.14(-0.17,0.44)	0.372
Metabolic status in obesity				
MHO(ref)				
MUO	-0.03(-0.57,0.50)	0.904	-0.19(-0.97,0.60)	0.627
Models were adjusted for lifestyle factors (smoking, drinking and physical activity), BMI, PBF and WHR.				
Abbreviations: HOMA-IR = homeostasis model assessment of insulin resistance; hsCRP = high-sensitivity CRP; MH = metabolically healthy; MU = metabolically unhealthy; MHO = metabolically healthy obesity; MUO = metabolically unhealthy obesity; BMI = body mass index; PBF = percent body fat; WHR = waist hip ratio.				

With respect to serum hsCRP level, no significant associations were found with metabolic status or number of MetS component in both MZ and DZ twin-pair analysis.

Results of obesity-metabolic categories with insulin resistance, and chronic inflammation level were shown in Table 5. After adjustment for lifestyle factors and shared genetic and familial factors in MZ twin-pair analysis, the level of HOMA-IR was higher in MUNO (β = 0.42,95% CI: 0.21–0.64, p < 0.001), MHO (β = 0.68,95% CI: 0.36–1.00, p < 0.001) and MUO (β = 0.69,95% CI: 0.46–0.91, p < 0.001) twins, compared with their MHNO counterparts. Twins with MHO or MUO had a significantly higher level of hsCRP compared with their MHNO counterparts. No significant differences were observed between MUNO and MHNO twins with respect to hsCRP level.

Table 5
Results of fixed linear regression models on the association of serum HOMA-IR and hsCRP levels with obesity-MetS categories in twin pairs

Exposure	MZ		DZ	
	β (95% CI)	p	β (95% CI)	p
HOMA-IR				
Obesity–MetS phenotype				
MHNO (ref)				
MUNO	0.42(0.21,0.64)	< 0.001	0.56(0.30,0.82)	< 0.001
MHO	0.68(0.36,1.00)	< 0.001	0.51(0.17,0.85)	0.003
MUO	0.69(0.46,0.91)	< 0.001	1.05(0.79,1.32)	< 0.001
hsCRP				
Obesity–MetS phenotype				
MHNO (ref)				
MUNO	0.21(-0.15,0.58)	0.249	0.33(-0.01,0.67)	0.061
MHO	0.74(0.21,1.27)	0.007	0.51(0.06,0.95)	0.025
MUO	0.54(0.16,0.93)	0.006	0.66(0.32,1.01)	< 0.001
Models were adjusted for lifestyle factors (smoking, drinking and physical activity).				
Abbreviations: MZ = monozygotic; DZ = dizygotic; HOMA-IR = homeostasis model assessment of insulin resistance; hsCRP = high-sensitivity CRP; MetS = metabolic syndrome; MHO = metabolically healthy obesity; MUO = metabolically unhealthy obesity; MHNO = metabolically healthy non-obesity; MUNO = metabolically unhealthy non-obesity.				

Discussion

Using this Chinese twin sample controlling for genetic and familial influence, we found that metabolic status were independently associated with higher insulin resistance (IR). Serum HOMA-IR level was progressively higher in MHO, MUNO, and MUO as compared with MHNO phenotype and the highest level was seen in participants with both obesity and 4 MetS components. Within twin-pair analysis indicated there might exist common genetic influence between HOMA-IR and MHO/MUO phenotype. The chronic inflammation level, evaluated by hsCRP was similar among obese twins with various number of MetS components, which differed significantly to MHNO. This demonstrates that the development of chronic inflammation might closely relate to central obesity but not MetS.

High correlation of insulin metabolism and metabolic health status was established in present study, with a significantly graded relationship between the number of the MetS components and IR. Compared with MHNO phenotype, serum HOMA-IR level was progressively higher in MUNO, MHO and MUO group showing the simultaneous contributions of MetS and obesity to IR. It was postulated MHO group would be defined as a subgroup of obese individuals with an intermediate phenotype between MHNO and MUO individuals. This observation was in accordance with previous studies which indicated that insulin resistance indices are elevated in MetS compared to the individuals without MetS(23, 31). A cross-sectional study of 405 participants showed insulin resistance are elevated in MetS compared to the normal population(31). Obesity is a well-established risk factor for IR, and previous studies indicated IR plays a major role in the pathogenesis of cardiometabolic disorders and is a common consequence of ectopic accumulation of visceral fat and intracellular lipid (32–34). When adjusted for obesity indicators, the association between metabolic status and insulin resistance attenuated, but remained statistically significant. Besides, we found the number of MetS components correlated with IR regardless of obesity status. All these results indicated that there exist an independent relationship between MetS and insulin resistance besides the effect of obesity.

In the past decade it has become increasingly clear that persons with similar fat mass may present with completely distinct clinical metabolic profiles(35, 36). Similarly, we found a higher HOMA-IR levels of MUO with MHO group. This was consistent to previous studies conducted in obese adults (23, 37–39). In a study of 1458 adults from two independent populations, individuals with MUO had significantly higher level of HOMA-IR than MHO group(23). Another cross-sectional study of 3844 Spanish Caucasian adults showed the same results(39). The prevalence of MHO in our cohort of obese twins was 20.3% and data of different authors showed that the prevalence of this phenomenon in obese individuals varies widely from 6.0–38.4%(35). Evidence existed that lifestyle habits might partly explain the heterogeneity of obesity in terms of metabolic abnormalities. In this study, MHO phenotype occurred more frequently in younger and female twins, and were more likely to exercise and were less likely to smoke or drink. Mechanistically, lifestyle might modulate whole-body energy metabolism and insulin sensitivity. Recently, genome-wide association studies identified a set of loci harboring genes possibly controlling both body extra fat distribution (associated with IR) and the metabolic profile of excess adiposity (i.e., MHO or MUO). This was in according with our within twin-pair analysis. The difference of HOMA-IR was only significant between two

extreme metabolic phenotypes (0 vs. 4 MetS components) in obese MZ twins, and the difference of HOMA-IR between MHO and MUO was less in MZ twins than DZ twins. These observations suggested a common genetic influence exist, therefore the difference between the MHO and MUO phenotype may be partly attributed to specific genetic traits modulating body fat distribution in different regional fat depots which hold diverse biological properties and functions(40).

We found similar hsCRP levels between MHO and MUO group, which differed significantly to MHNO, and the association of numbers of MetS components with serum hsCRP began no more significant in the model additionally adjusted for obesity indicators. Studies conducted in Mitchelstown cohort participants (41), Wielkopolska general population(42) and a large sample of Brazilian population(22) all reported a similar level of CRP between MHO and MUO group. A study using six sets of criteria to define MHO found no significant difference of CRP with MUO subjects after multivariate analysis (43). Inconsistent findings have also been reported that MHO participants had significantly lower CRP levels than non-MHO(20, 21, 44). However, in most studies the difference in CRP levels between MHO and non-MHO subjects became no more statistical significance after adjusting for abdominal obesity or percent body fat(43, 44) which was in accordance with our finding. These results suggested that abdominal obesity per se is the key role in the progress of subclinical vascular inflammation. Obesity in absence of metabolic risk factors is not entirely benign and MHO population would therefore be expected to have a higher risk of CVD and all-cause mortality than MHNO group(45)

The strengths of our study include a twin design, a standard definition of MetS, adjustment for many available covariates, and various analyses including using stratified analysis and number of MetS components to test the robustness of the conclusions. However, several potential limitations should be addressed. First, the cross-sectional design can only address associations and not casual relationships. Second, the possibility of residual confounding by unmeasured covariates cannot be excluded. Finally, the gold standard for measurement of insulin resistance is the hyperinsulinemic-euglycemic glucose clamp technique. However, this is a research technique with limited clinical applicability and most studies use HOMA-IR as a clinically useful surrogate measure of insulin resistance.

Conclusion

In conclusion, our findings demonstrated that metabolic status was independent related to insulin resistance and the highest level was observed in individuals with both obesity and 4 MetS components. Within twin-pair analysis indicated there might exist common genetic influence between HOMA-IR and MHO/MUO phenotype. Higher inflammation level found among MU subjects might partly be related to abdominal obesity. These findings provide potential evidence for the different risk assessment based on metabolic status in obese population.

Declarations

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgements

We thank the participants and CDC twin project staff in Jiangsu Province, Zhejiang Province, Shandong Province and Sichuan Province for their invaluable contributions to this study.

Author Contributions

CX L was mainly responsible for the design, analyses and drafting of the manuscript. LM L was the principle investigator of this study. WJ G contributed to the conception and design of the study, oversaw the consenting and participated in manuscript polishing. CW H, J L, and SF W contributed to the conception and design of the study and interpreting the findings. CQ Y participated in data management and cleaning. ZC P, LM C, HW and XP W contributed to the implementation of the study. All authors contributed to the interpretation of results and intellectual content of the manuscript, and have read and approved the final manuscript.

Funding information

This study was supported by Special fund for Health scientific research in the public welfare (201502006, 201002007), and Key Project of Chinese Ministry of Education (310006).

Ethics approval and consent to participate

Biomedical Ethics Committee at Peking University, Beijing, China approved the study protocol. An informed consent was obtained from each participant. Data were treated with strict confidentiality and used only for scientific purposes.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

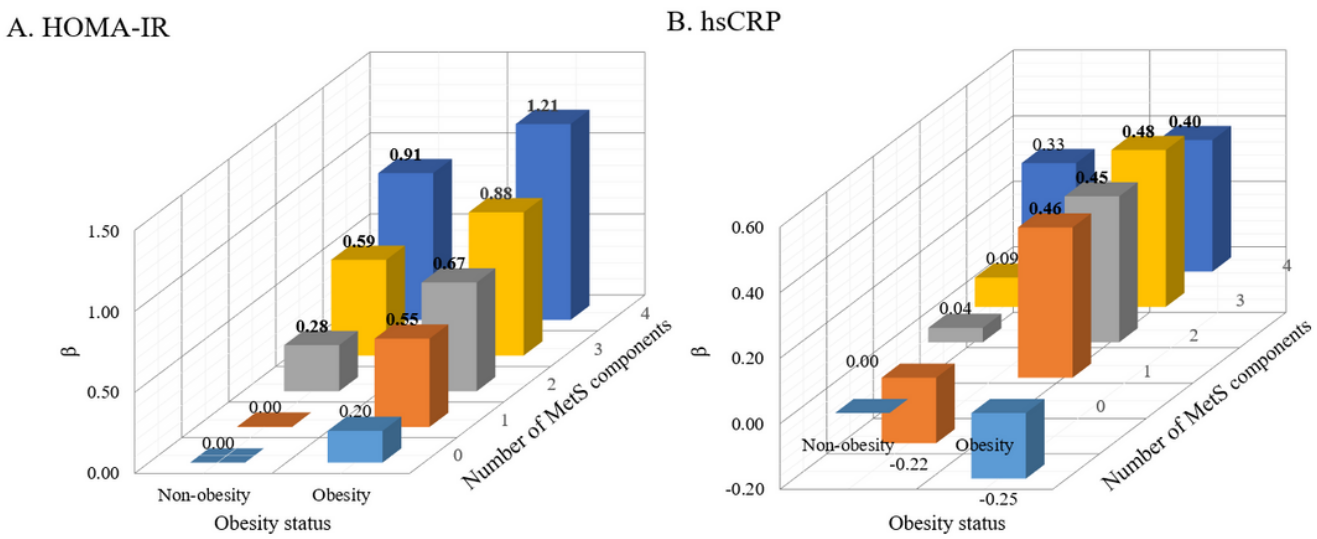

Figure 1

(A) Association between obesity status, number of MetS components and level of HOMA-IR (B) Association between obesity status, number of MetS components and level of hsCRP. The bolded number means the correlation was significant at p<0.05. The adjusted covariates included sex, zygosity, place, age, lifestyle factors (smoking, drinking, and physical activity), TC and HA1bC. Abbreviations: MetS = metabolic syndrome; HOMA-IR=homeostasis model assessment of insulin resistance; hsCRP=high-sensitivity CRP.

Supplementary Files

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- Supplementarytables.pdf