ORIGINAL RESEARCH

Associations of Metabolic/Obesity Phenotypes with Insulin Resistance and C-Reactive Protein: Results from the CNTR Study

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Background: Obesity is a heterogeneous condition in terms of metabolic status. Different obesity phenotypes have various health risks. The aim of this work was to define different subtypes of obesity and investigate their relationship with inflammatory-cardiometabolic abnormalities 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. We defined those with 0 or 1 metabolic syndrome (MetS) components excluding waist circumference as metabolically healthy, and those with waist circumference ≥90 cm (for men) and ≥85 cm (for women) as obese. The two-category obesity status and metabolic states are combined to generate four metabolic/obesity phenotypes. 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. Mixed-effect linear regression models and fixed-effect linear regression models were used to analyse the correlation between HOMA-IR, hsCRP and different metabolic/obesity phenotypes.

Results: In cross-sectional analyses of 1113 individuals (mean [SD] age, 46.6 [12.9] years; 463 obese [41.6%]), 20.3% obese twins were metabolic healthy and 64.2% non-obese twins were metabolic unhealthy. Serum HOMA-IR level was higher in metabolically unhealthy non-obesity (MUNO) (β=0.42, 95% CI: 0.21–0.64), metabolically healthy obesity (MHO) (β=0.68, 95% CI: 0.36–1.00) and metabolically unhealthy obesity (MUO) (β=0.69, 95% CI: 0.46–0.91) twins, compared with their metabolically healthy non-obesity (MHNO) counterparts. HsCRP was similar between MHO and MUO, which differed significantly to metabolic healthy non-obesity (MHNO).

Conclusion: MHO and MUNO phenotypes were common in Chinese twin population. Both phenotypes were associated with elevated IR and hsCRP which may not be benign and need to be concerned.

Keywords: metabolic status, insulin resistance, chronic inflammation, twin study

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Introduction

There is clear evidence of an association between obesity and various metabolic abnormalities such as insulin resistance, inflammation, metabolic syndrome (MetS), which increases the risk of type 2 diabetes mellitus (T2D), and of various cardiovascular outcomes.[1,2] However, not all obese individuals entail metabolic abnormalities. These obese individuals who display a healthier phenotype have been called metabolically healthy obesity (MHO).[3] In general, the risks of T2D, cardiovascular

diseases (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),[2,4–7] although inconsistent results have also been reported.[8,9] Furthermore, recent studies have found that the risks of cardiovascular outcomes are directly related to the number of metabolic abnormalities,[10–13] but the underlying mechanism is not clear yet.

Insulin resistance and inflammation are the potential mechanism linking obesity and cardiometabolic risk.[14,15] Insulin resistance and inflammation can result in hyperglycemia, hypertension, dyslipidemia and contribute to cardiometabolic syndrome and increased CVD risk.[16,17] Studies have found that insulin sensitivity is better in MHO individuals when compared to MUO individuals, but worse than those who are MHNO.[18–20] To date findings have been inconsistent about the relationship between inflammation level and MHO/MUO phenotype. Some studies reported that MHO participants had significantly lower CRP levels than MUO individuals,[21,22] but other studies found a similar level of CRP between the two groups.[23,24] These estimates are mostly based on studies of Western populations, with little evidence from Chinese populations.

The factors responsible for the different metabolic phenotypes of obesity are not clear, but differences in potentially modifiable lifestyle factors and genetic factors associated with adipose tissue biology may play an important role. Genome wide association studies (GWAS) have identified genetic variants that are associated with both adiposity and metabolic profile.[25] It was possible that the previous associations found between metabolic abnormalities and clinical characteristics were attributable to common genetic vulnerabilities. Twin design, especially monozygotic (MZ) twins who are completely matched for genetic background, have an extremely powerful ability to control genetic factors.

Therefore, it is necessary to clarify the insulin resistance and inflammation level in different metabolic/obesity groups which have profound importance in assessing and managing high-risk obesity. In this study, we aimed to define different metabolic/obesity phenotypes and investigate their relationship with insulin resistance and inflammation in a subcohort of Chinese National Twin Registry (CNTR).

Methods
Study Population
The study population was a subcohort of CNTR, which is a cross-sectional study of 1147 adult twins aged 18 to 79 years, recruited between April to December 2013 from four provinces in China (Shandong, Zhejiang, Jiangsu and Sichuan province). Details of CNTR design and survey methods have been previously described.[26] All participants in this study completed an interviewer-administered questionnaire, underwent a physical examination and provided a fasting blood sample.

Twins were excluded from analyses if:[1] with a definitive diagnosis of alimentary tract tumor, cardiovascular heart disease, stroke and kidney disease;[2] using weight-loss drug in the last month. As a result, 34 individuals were excluded, leaving 1113 individuals (541 completed twin pairs and 31 individual twins) for this study. Among 541 twin pairs, 35 twin pairs were raised separately for an average of seven years (range 1–11 years, reared apart was defined according to SATSA's definition as twins who had been reared apart for at least 1 year before the age of 11).

Determination of zygosity was based on the information from questionnaires during the baseline investigation conducted in 2010. This has been validated using DNA genotyping and found to be >90% accurate.[27] This study was approved by Biomedical Ethics Committee at Peking University, Beijing, China. All participants provided their written informed consent.

Clinical and Biochemical Data Collection
Information on demographic characteristics, medical history, and lifestyle factors were recorded by questionnaire, including questions on tobacco smoking, alcohol drinking and exercise activities. Each participants' occupation, transportation, daily life and leisure time exercise activities was assigned a metabolic equivalent task (MET) value[28] and we calculated the total MET value in the analysis.

Each participant's blood pressure, height, weight and percent body fat (PBF) were measured. Blood pressure was calculated as the mean of the three consecutive measurements. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference (WC) was measured three times at the level of the umbilicus and we calculated the mean value in the analyses. PBF was determined by bioelectrical impedance (Body Composition Analyzer/Scale, TANITA).

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Venous serum blood samples were collected after 12h fasting for detection of serum lipid indicators including total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol, serum glucose indicators including glucose (Glu) and serum insulin. 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) concentration was measured by a high-sensitivity immunoturbidimetric method using Hitachi auto-analyzer (Roche Diagnostics, Mannheim, Germany). Details of these biochemical data collection have been previously described.[29]

Definition of the Phenotypes

Metabolic status was defined according to ATP-III components:[30] 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 drugs; 3) HDL cholesterol <1.0 mmol/L (in men) and <1.3 mmol/L (in women) or using lipid-lowering drugs; and 4) triglycerides ≥1.7 mmol/L or using lipid-lowering drugs. Participants with ≤1 abnormal component were defined as metabolically healthy (MH), with the remaining defined as metabolically unhealthy (MU). Individuals with waist circumference ≥90 cm (men) and 85 cm (women) were considered obese.[31] Metabolic and obesity status were combined to create the four metabolic/obesity phenotypes: metabolically healthy non-obesity (MHNO), metabolically healthy obesity (MHO), metabolically unhealthy non-obesity (MUNO), metabolically unhealthy obesity (MUO).

Statistical Methods

We used mean ± SD or median (first quartile (Q1) – third quartile (Q3)) for continuous variables and absolute frequencies for categorical variables. We compared epidemiological and clinical 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.

We used mixed-effect linear regression models which take into account for twin clustering to assess the relationship of serum HOMA-IR and hsCRP levels with metabolic status (MH as reference group), number of MetS components, and the combined metabolic/obesity categories (MHNO as reference group), adjusting for various covariates. The first model was adjusted for age, sex, place, and zygosity; the second model was additionally adjusted for lifestyle factors (smoking status, drinking status, and METs level), and obesity indicators or serum clinical measurements were further adjusted in the third model where appropriated. Further, we repeated the analysis stratified by age (group1: age<45, group2: age≥45). To better control for shared genetic and environmental factors, we used within twin-pair fixed-effect linear regression model. Twins reared together are matched for age, genetic background, early intrauterine environment and family upbringing environment. This within twin-pair analysis approach automatically takes into account these matched factors. 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). Sensitivity analysis was conducted in twins reared together (excluding 35 twin pairs reared apart). The statistical analysis was performed with Stata statistical software (release 12.0; Stata Corporation, College Station, TX, USA). We logarithmic transformed all the clinical indicators in the analyses. Significance were assessed at $P<0.05$ (two-sided).

Results

Define the Metabolic/Obesity Phenotypes

This analysis included 1113 participants (46.55 ± 12.89 years, 65.4% men), containing 541 complete twin pairs (336 MZ twin pairs, 205 DZ twin pairs). 41.6% twins were obese with 20.3% obese twins were metabolic healthy. Among non-obese twins, 35.8% were metabolic healthy with the remaining in the MUNO group (Table 1). The MHO twins had a mean age of 40.85 ±12.03 years with 52.1% women. When comparing MHO to the MUO group twins, we found lower WC, WHR (p<0.0001) and lower concentrations of most biochemical characteristics except hsCRP in MHO group. Besides, the MHO group had the lowest proportion of current smokers (18.1%) and current drinkers (16.0%)

Table 1 Baseline Characteristics of the Study Participants (N = 1113)

Characteristics	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 activities, 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
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

Note: Data are presented as means (SD)/median (IQR) for continuous variables and numbers (percentage) for categorical variables.
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.

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.

Metabolic Status with Insulin Resistance and hsCRP

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 adjusting for lifestyle factors (smoking, drinking, and physical activity) and obesity indicators (model 3), the associations remained significant though 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).

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. Test for trend indicated a dose-response relation between numbers of MetS components and serum HOMA-IR (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).

Metabolic/Obesity Phenotypes with Insulin Resistance and hsCRP

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 was associated with the minimum increase in serum HOMA-IR and hsCRP levels. After adjusting for lifestyle factors (smoking status, drinking status, and MET values), TC and HbA1c (model 3), all the associations were still significant though there were a slight decrease (Table 3). Analysis stratified by age did not find a significant

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

Notes: Model 1 was adjusted for sex, zygosity, place and age; model 2 was adjusted for model 1 plus lifestyle factors (smoking status, drinking status, and MET values); 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.

Table 3 Results of Mixed Linear Regression Models on the Association of Serum HOMA-IR and CRP Levels with Metabolic/Obesity Phenotypes 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/obesity 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						
Metabolic/obesity 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

Notes: Model 1 was adjusted for sex, zygosity, place and age; model 2 was adjusted for model 1 plus lifestyle factors (smoking status, drinking status, and MET values); model 3 was adjusted for model 2 plus TC and HA1bc.
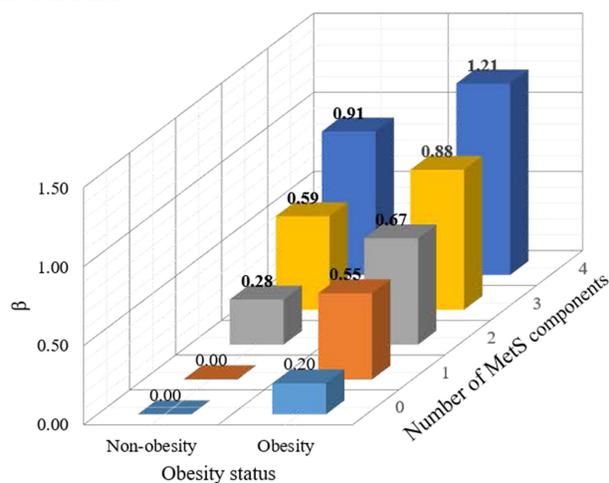**Abbreviations:** HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity CRP; MetS, metabolic syndrome; MHO, metabolically healthy obesity; MUO, metabolically unhealthy obesity; MHNO, metabolically healthy non-obesity; MUNO, metabolically unhealthy non-obesity; TC, total cholesterol.

interaction between metabolic/obesity phenotypes and age (Table S4).

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 (Figure 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 MHUO group, participants with obesity and any MetS component had similar higher levels of hsCRP.
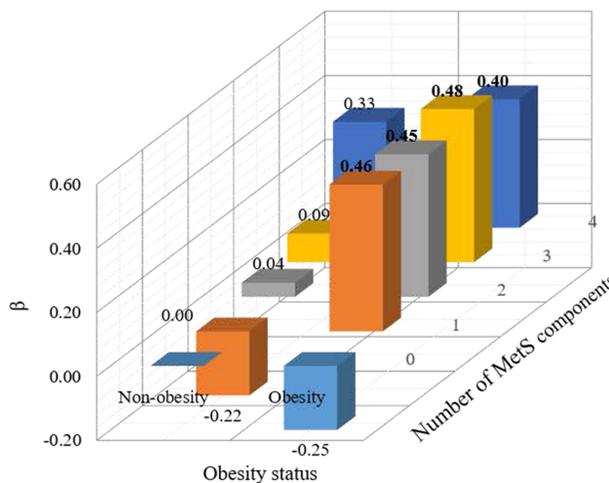
A. HOMA-IR

B. hsCRP

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.

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

Note: Models were adjusted for lifestyle factors (smoking status, drinking status, and MET values), 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.

Within Twin-Pair Analysis

In within twin-pair analyses controlling for genetic and familial influences among 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. With respect to serum hsCRP level, no significant associations were found between hsCPR with metabolic status in both MZ and DZ twin-pair analysis. Sensitivity analysis excluding 35 twin pairs reared apart did not change the results (Table S5).

Similarly, we analyzed the associations of number of MetS components with serum HOMA-IR and hsCRP levels (Table S6). 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 significantly higher level of serum HOMA-IR (β=1.01,95% CI: 0.07–1.96, p = 0.036, Table S6). No significant associations were found between hsCPR with number of MetS components in both MZ and DZ twin-pair analysis.

Results of metabolic/obesity phenotypes with insulin resistance, and chronic inflammation level are 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. Sensitivity analysis excluding 35 twin pairs reared apart did not change the results (Table S7).

Discussion

In this Chinese twin sample, we found that obesity is a heterogeneous condition. A certain proportion of obese individuals have a normal metabolic profile while a number of non-obese adults present with a clustering of metabolic abnormalities. Serum HOMA-IR level was higher in MUO, MHO, and MUNO as compared with

Table 5 Results of Fixed Linear Regression Models on the Association of Serum HOMA-IR and hsCRP Levels with Metabolic/Obesity Phenotypes in Twin Pairs

Exposure	MZ		DZ	
	β (95% CI)	*p*	β (95% CI)	*p*
HOMA-IR				
Metabolic/obesity 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				
Metabolic/obesity 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

Note: Models were adjusted for lifestyle factors (smoking status, drinking status, and MET values).
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.

MHNO group and the highest level was seen in twins with both obesity and 4 MetS components. The inflammation level, evaluated by hsCRP was similar among obese twins with various number of MetS components, which differed significantly to MHNO.

We showed that in this study, MHNO and MHO individuals were younger, with a higher proportion of women, and had lower rates of smoking and drinking than MUNO and MUO phenotype. Evidence existed that lifestyle factors might play an important role in the heterogeneity metabolic status of obesity through modulating whole-body energy metabolism and insulin sensitivity.[32] 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% to 38.4%.[33]

Significant correlation of insulin metabolism and metabolic health status was found in present study, with an obvious linear trend between the number of the MetS components and IR. Compared with MHNO phenotype, serum HOMA-IR level increased gradually in MUNO, MHO and MUO group showing the simultaneous contributions of MetS and obesity to IR. Significant results also exist in reared together MZ twin pairs where genetic background, early intrauterine environment and family upbringing environment were perfectly matched. Our findings confirm those results conducted in the general

population which indicated that insulin resistance indices are elevated in MetS compared to the individuals without MetS.[24,34] A cross-sectional study of 405 participants showed insulin resistance are elevated in MetS compared to the normal population.[34] Obesity is a well-established risk factor for IR, and previous studies indicated IR plays a major role in the pathogenesis of cardiometabolic diseases and is a common consequence of ectopic accumulation of visceral fat and intracellular lipid.[35–37] 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.

In the past decade it has become increasingly clear that persons with similar fat mass may present with completely distinct clinical metabolic profiles.[33,38] Similarly, we found a higher HOMA-IR levels of MUO with MHO group. This was consistent to previous studies conducted in obese adults.[24,39–41] In a study of 1458 adults from two independent populations, individuals with MUO had significantly higher level of HOMA-IR than MHO group.[24] Another cross-sectional study of 3844 Spanish Caucasian adults showed the same results.[41] Recently, genome-wide association studies identified serval genes possibly controlling both body extra fat distribution and the metabolic profile of excess adiposity (ie, MHO or MUO).[42] This was in

according with our within twin-pair analysis. The difference of HOMA-IR was no longer significant between MHO and MUO phenotypes in obese MZ twin pairs but remained significant in DZ twin pairs. These observations suggested a common genetic influence existed between insulin sensitivity and MHO/MUO phenotype. Examining twins overall gives an average relationship between exposure and outcome across the twin population. If the association further persists in within MZ twin-pair comparisons we can infer that something unique to each individual twin is contributing, rather than common to both twins. On the contrary, attenuation of the association in MZ twin pairs indicated that it was confounded by genetic factors.

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,[43] Wielkopolska general population[44] and a large sample of Brazilian population[23] 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.[45] Inconsistent findings have also been reported that MHO participants had significantly lower CRP levels than MUO.[21,22,46] However, in most studies the difference in CRP levels between the two groups had no more statistical significance after adjusting for abdominal obesity or percent body fat,[45,46] 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 have a higher risk of CVD and all-cause mortality than MHNO group.[47]

Our research has the following advantages. First, we applied a twin design to control for genetic and familial factors, and central obesity was used instead of general obesity in the analysis. Furthermore, we conducted various analyses including stratified analysis by age and excluding twins reared apart to ensure the robustness of the associations. However, our study had several limitations as well. First, the cross-sectional design restricted the ability of causal inference. Second, although WC might be a better indicator for abdominal adiposity than BMI, it cannot accurately reflect visceral fat content which is closely

related to metabolic status. Finally, we did not use the gold standard for measurement of insulin resistance. However, most studies use HOMA-IR as a clinically useful surrogate measure of insulin resistance.

Conclusion

In conclusion, our findings demonstrated that obesity is a heterogeneous condition in terms of metabolic status. MHO and MUNO phenotypes are common in these Chinese twin population. Insulin resistance and inflammation level displayed various levels among different metabolic/obesity groups. MHO and MUNO phenotypes were associated with elevated IR and hsCRP and individuals with both obesity and 4 MetS components had the highest level of insulin resistance. These findings provide potential evidence for assessing and managing high-risk obesity based on metabolic status.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki. 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.

Disclosure

The authors declare that they have no competing interests.

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