DOI: 10.4274/tjem.3600
Turk J Endocrinol Metab 2016;20:117-122

Endothelial Function in Distinct Phenotypes of Obesity

Farklı Obezite Fenotiplerinde Endotelyal Fonksiyon

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Abstract

Purpose: In our study, we aimed to determine whether metabolically healthy subjects with obesity would show endothelial dysfunction (ED) when compared with insulin-resistant subjects with obesity.
Material and Method: We enrolled 231 subjects with obesity (83% female) in this cross-sectional study. Brachial artery flow-mediated dilation was performed by Doppler ultrasonography and a standard 75-g oral glucose tolerance test were carried out in all participants. The subjects were stratified into tertiles based on their insulin sensitivity index values and defined as having insulin-resistant obesity if the values were in the lower tertile (n=77) or metabolically healthy obesity if the values were in the upper tertile (n=77). ED was defined as Δ flow-mediated dilation <4.5%.
Results: Metabolically healthy obesity and insulin-resistant obesity groups had similar ages (39±9 vs. 40±10 years; p=0.59) and body mass index (38±5 vs. 39±5 kg/m^2; p=0.09). Waist circumference (101±11 vs. 106±13 cm; p=0.01), fasting blood glucose (87±9 vs. 97±13 mg/dL; p<0.001), diastolic blood pressure (79±11 vs. 82±12 mmHg; p=0.04) and uric acid levels (4.6±1.0 vs. 5.3±1.3 mg/dL; p<0.001) were lower in metabolically healthy obesity subjects, however, the incidence of ED was similar in both metabolically healthy obesity and insulin-resistant obesity subjects (80% vs. 71%; p=0.25, respectively).
Discussion: The incidence of ED, assessed by flow-mediated dilation, was similar both in metabolically healthy obesity and insulin-resistant obesity subjects. In this study, we showed that subjects with obesity as defined as metabolically healthy obesity might also show ED.
Keywords: Obesity, insulin resistance, flow-mediated dilatation

Öz

Amaç: Çalışmamızda metabolik olarak sağlıklı obez bireylerle, insülin direnci olan bireylerin endoteyal fonksiyonlarını (ED) değerlendirmeyi amaçladık.
Gereç ve Yöntem: Bu kesitsel çalışmaya 231 obez birey (%83 kadın) alınmıştır. Katılımcıların brakial arterinde Doppler ultrasonografi ile akım aracılı genişleme değerlendirildi ve tüm katılımcılara 75 gram oral glukoz tolerans testi uygulandı. Katılımcılar insülin sensitivite indekslerine göre üç gruba ayrıldı ve insülin duyarlılık indeksi değerleri alt tertildeki (n=77) bireyler insülin resistant obez grup olarak ve üst tertildeki obezler (n=77) metabolik olarak sağlıklı obez grup olarak değerlendirildi. ED Δ akım aracılı dilatasyon <%4,5 olarak tanımlandı.
Bulgular: Metabolik olarak sağlıklı obez grup ve insülin resistant obez grupları benzer yaş (39±9 vs. 40±10 yıl; p=0,59) ve vücut kitle indeksine (38±5 vs. 39±5 kg/m^2; p=0,09) sahiptiler. Bel çevresi (101±11 vs. 106±13 cm; p=0,01), açlık kan şekeri (87±9 vs. 97±13 mg/dL; p<0,001), diyastolik kan basıncı (79±11 vs. 82±12 mmHg; p=0,04) ve ürik asit düzeyleri (4,6±1,0 vs. 5,3±1,3 mg/dL; p<0,001) metabolik olarak sağlıklı obez grup grubunda daha düşüktü bununla birlikte ED görülme sıklığı her iki grupta benzerdi (%80 vs. %71; p=0,25, sırasıyla).
Tartışma: ED görülme sıklığı metabolik olarak sağlıklı değerlendirilen obez bireylerde insulin direnci olan obez bireylerdeki gibidir. Çalışmamızda metabolik olarak sağlıklı değerlendirilen obez grubun da ED'yi gösterdiği tespit edilmiştir.
Anahtar kelimeler: Obezite, insülin direnci, akım aracılı dilatasyon

Introduction

The worldwide prevalence of obesity has more than doubled and it has become a growing public health problem (1). Undesirable conditions, such as type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD), may accompany obesity, which may lead to a higher incidence of all-cause mortality. However, not all obese subjects show the same metabolic profiles. Some of them may exhibit a more favorable, healthy profile, which is characterized by high insulin sensitivity levels, favorable lipid profiles, satisfactory fat distribution, low hypertension incidence and low systemic inflammatory marker levels; (2) this has been termed as metabolically healthy obesity (MHO) (3). MHO subjects constitute 10-50% of all obese people on the basis of the criteria used to define MHO subjects based on the results reported by

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different studies (4). It is likely that the MHO phenotype is the result of several underlying mechanisms and the interactions between genetic, environmental and behavioral factors. Each of these factors affect abdominal fat distribution, visceral and ectopic fat accumulation and insulin resistance (IR), which are all important causative factors that contribute to the development of unhealthy obesity (2,5). In some cases, the concept of the MHO phenotype is used interchangeably with the notion of insulin-sensitive obesity because MHO subjects display better insulin sensitivity than metabolically unhealthy subjects (2,6).

Endothelial dysfunction (ED) is the main vascular event in atherogenesis and precedes the development of clinically detectable atherosclerotic plaques in coronary arteries (7). IR triggers ED, and together they play a central role in the pathogenesis of atherosclerosis (8,9). Insulin is not only a metabolic hormone but also has vascular haemodynamic actions. It has a direct vasodilatory effect mediated through the stimulation of nitric oxide (NO) production in endothelial cells. Endothelial-derived NO deficiency is believed to be the primary defect that links IR and ED (10,11). ED limits the ability of insulin to reach its target organ (12). Reduced expansion of the capillary network with attenuation of microcirculatory blood flow to metabolically active tissues contributes to the impairment of insulin-stimulated glucose and lipid metabolism (2,9,13,14). This results in a recurring negative feedback cycle in which ED and disturbances in glucose and lipid metabolism develop secondary to IR (9). Vascular damage, which results from lipid deposition and oxidative stress to the vessel wall, triggers an inflammatory reaction and the release of chemoattractants and cytokines, which worsens IR and ED (9,15,16,17). Endothelial function may be assessed by measuring plasma levels of markers, such as soluble vascular cell adhesion molecule, soluble intercellular adhesion molecule, E-selectin, endothelin-1, plasminogen activator inhibitor-1, von Willebrand factor, C-reactive protein (CRP), interleukin-1 and -6 and tumor necrosis factor alpha (TNF-α) (8,9). A commonly used and non-invasive way to evaluate endothelial function is the assessment of flow-mediated dilation (FMD) by performing an ultrasound in the brachial artery (18,19).

Evidence supports the relationship between IR and abdominal obesity as well as that between IR and ED, but studies analyzing the specific role of IR in ED associated with obesity are lacking. In this study, we aimed to determine whether two distinct obesity phenotypes (insulin sensitive vs. insulin resistant) with similar body mass index (BMI) would show different vascular functions when analyzed with FMD.

Materials and Methods

Study Population

In this study, the prospectively generated database of the obesity polyclinic (a single centre, cross-sectional design) was analyzed. Two hundred thirty one obese subjects (aged 18-65 years), who attended the obesity outpatient clinic at Kartal Dr. Lütfi Kırdar Training and Research Hospital in İstanbul between June 2013 and January 2015, were evaluated. The physical and biochemical test records of the subjects at first admission to the obesity outpatient clinic were examined. Subjects with chronic illnesses (cancer, chronic renal failure, chronic liver disease, and pulmonary, psychiatric, inflammatory and/or infectious diseases) and/or endocrine diseases (such as diabetes mellitus, thyroid dysfunction and Cushing's syndrome) were excluded. Subjects using medication were also excluded. The study was conducted in agreement with the Declaration of Helsinki II. Kartal Dr. Lütfi Kırdar Training and Research Hospital Ethics Committee approved the study protocol, and informed consent was obtained from all subjects.

Design

Subjects were evaluated for specific data at first admission: age, gender, history (presence of comorbidities and medication use), physical examination [weight (kg), height (m)], BMI (kg/m^2) and waist circumference (WC) (cm). Blood pressure was measured in the right arm using an automated sphygmomanometer after the patient rested for 5 min. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded.

Blood tests were performed after overnight fasting. Biochemical and hormonal parameters [fasting blood glucose (FBG), fasting insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoproein cholesterol (LDL-C), triglyceride (TG), uric acid levels, creatinine, thyroid-stimulating hormone (TSH), 25-hydroxyvitamin D [25(OH)D], CRP and white blood cell (WBC) counts] were examined concurrently at first admission. Study subjects underwent a standard 75-g oral glucose tolerance test (OGTT). Blood samples were taken at 0, 30, 60, 90 and 120 min for the assessment of blood glucose and insulin concentrations. IR was calculated by the homeostasis model assessment of IR (HOMA-IR) and insulin sensitivity index (ISI) using the following formulas: HOMA-IR=[fasting plasma insulin (μIU/mL) × fasting plasma glucose (mg/dL)] / 405 and ISI=[10.000/square root of (fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT)]. Because different levels of insulin sensitivity are determined by different indices, it is difficult to determine an objective definition of IR. Therefore, the sample study group (n=231) was stratified into tertiles based on their ISI values, and the subjects were defined as having insulin-resistant obesity (IRO) if the values were in the lower tertile (n=77) or MHO if the values were in the upper tertile (n=77).

The presence of metabolic syndrome (MS) was assessed according to the definition of the National Cholesterol Education Program-Adult Treatment Panel III as the presence of three or more of the following features: abdominal obesity (WC of ≥88 cm in women and ≥102 cm in men); hypertriglyceridemia (≥150 mg/dL); low HDL-C (<40 mg/dL in men or <50 mg/dL in women); hypertension (SBP ≥130 mmHg or DBP ≥85 mmHg or ongoing antihypertensive therapy) and hyperglycemia (≥100 mg/dL).

Laboratory Analysis

Plasma venous glucose was measured using the hexokinase method. Serum insulin levels were measured by the immunoassay method (Abbott Diagnostics, USA). Serum uric acid, TC, HDL-C, TG levels were measured by using enzymatic calorimetric methods (Beckman Coulter Inc. USA). LDL-C was

calculated using Friedewald formula [LDL-C=TC-(TG/5 + HDL-C)]. TSH was measured by chemiluminescence immunoassay (Beckman Coulter Inc., USA). 25(OH)D levels were measured by chromatography using high-performance liquid chromatography (Shimadzu Corporation, Japan). High sensitivity CRP was assayed by the nephelometric method.

Flow-Mediated Dilation

An experienced sonographer who was blinded to the patients' information obtained ultrasound images using a 7.5-MHz linear array transducer (Toshiba Aplio 300). FMD was measured as defined in previous studies (20,21). Measurements were performed in a silent, temperature controlled (20 °C) room. Subjects were fasted and rested for at least 10 min before the examination. After measuring the baseline diameter of the right brachial artery (1 cm segment, at 3 different points) on the antecubital fossa, the blood pressure cuff was placed on the right forearm and inflated up to 220 mmHg for 5 min. The post-ischemic scan was performed 45-60 sec after cuff deflation. The maximal brachial artery diameter was measured. FMD was determined as the percent diameter change of the post occlusion measurement to the baseline measurement. The percent FMD change (<4.5%) was considered as ED (20). To determine intraobserver variability, 10 patients underwent two independent measurements. Intraobserver variability was 4%.

Statistical Analysis

Data are presented as mean ± standard deviation for continuous variables or median (25% and 75% interquartiles) for non-normally distributed variables. Normality of data distribution was assessed by the Kolmogorov-Smirnov test. The tests of significance used were the independent sample t-test for normally distributed variables, the Mann-Whitney U test for non-normally distributed variables and chi-square test for categorical variables. The study population was divided into three BMI groups. Tests of significance used were the one-way ANOVA for normally distributed variables and the Kruskal-Wallis test for non-normally distributed variables. The subjects (n=231) were stratified into tertiles based on their ISI values, and they were defined as IRO if their ISI values were in the lower tertile (n=77) or MHO if the values were in the upper tertile (n=77). Logistic regression analysis was performed to determine independent predictors of the percent of ΔFMD in all participants. The Hosmer-Lemeshow goodness-of-fit statistics were used to assess the model fit. A 5% type 1 error level was used to infer statistical significance.

Results

General characteristics of the study population according to gender are given in Table 1. Two hundred thirty one obese subjects fulfilling the inclusion criteria were recruited for this study. 83% (n=192) of the study population were women. Mean age (female: 40±10 years and male: 40±12 years) and BMI (female: 38±5 kg/m^2 and male: 38±5 kg/m^2) were similar in both sexes. Metabolic parameters consisting of WC, SBP, DBP, and TG and uric acid levels were statistically significantly higher in men and HDL-C was higher in women. Median HOMA-IR [2.4 (1.6-3.5) vs. 2.7 (1.9-3.9); p=0.12] and ISI [4.0 (2.5-5.8) vs. 3.7 (2.3-4.9); p=0.11] values and presence of ED (75% vs. 77%), assessed by FMD, were similar in females and males, respectively.

BMI groups organized as group 1 (BMI=30-34.9; n=77); group 2 (BMI=35-39.9; n=74) and group 3 (BMI ≥40; n=80) are given in Table 2. Age and gender distribution was similar in all BMI groups. SBP, DBP, HOMA-IR, uric acid levels and CRP increased, whereas ISI decreased in parallel with the BMI increase. Lipid profiles, fasting and 2-h glucose levels and presence of ED were similar in all BMI groups, but MS was positive in 39% of patients in group 1, 43% in group 2 and 65% in group 3.

The groups organized according to ISI tertiles are given in Table 3. The lowest ISI tertile was named as IRO (n=77) and upper ISI tertile was named as MHO (n=77). Age, gender distribution and BMI were similar in IRO and MHO groups. 25(OH)D and TSH levels were similar in both groups. WC (101±11 vs. 106±13 cm; p=0.01),

Table 1. Anthropometric, main metabolic parameters and presence of endothelial dysfunction (Δ flow-mediated dilation <4.5%) of the study subjects and comparison by gender

	All subjects (n=231)	Female (n=192)	Male (n=39)	p
Age (year)	40±10	40±10	40±12	0.74
BMI (kg/m^2)	38±5	38±5	38±5	0.83
Current smoker (%)	18	20	11	0.17
Weight (kg)	98±15	96±14	110±18	<0.001
WC (cm)	103±12	101±11	112±12	<0.001
SBP (mmHg)	126±19	125±17	134±23	0.004
DBP (mmHg)	81±12	79±11	87±14	<0.001
ED (%) (ΔFMD<4.5%)	76	75	77	0.85
TC (mg/dL)	199±37	198±37	203±38	0.50
HDL-C (mg/dL)	47±9	48±9	42±6	<0.001
LDL-C (mg/dL)	126±32	125±31	131±32	0.24
TG (mg/dL)	118 (89-160)	114 (85-148)	137 (102-186)	<0.02
FG (mg/dL)	92±11	92±11	92±12	0.67
2-h glucose (mg/dL)	113±34	112±32	118±37	0.26
FI (μU/mL)	11 (8-16)	11 (8-16)	12 (9-19)	0.12
HOMA-IR	2.5 (1.7-3.7)	2.4 (1.6-3.5)	2.7 (1.9-3.9)	0.12
ISI	4.4 (2.5-5.6)	4.0 (2.5-5.8)	3.7 (2.3-4.9)	0.11
Uric acid (mg/dL)	4.9±1.1	4.6±0.9	6.0±1.3	<0.001
Crea (mg/dL)	0.67±0.22	0.64±0.22	0.81±0.17	<0.001
25(OH)D3 (ng/mL)	10 (7-15)	8 (6-13)	10 (7-15)	0.03
TSH (μIU/mL)	1.8 (1.3-2.9)	1.9 (1.3-3.2)	1.6 (1.3-2.0)	0.06
CRP (mg/L)	6.2 (3.5-9.9)	6.2 (3.4-9.4)	5.7 (3.5-10.1)	0.83
WBC (x10^3/mm^3)	7.7±1.7	7.6±1.7	8.1±2.0	0.12

BMI: Body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure ED: Endothelial dysfunction, TC: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoproein cholesterol, TG: Triglyceride, FI: Fasting insulin, HOMA-IR: Homeostasis model assessment of IR, ISI: Insulin sensitivity index, 25(OH)D3: 25-hydroxyvitamin D3, TSH: Thyroid-stimulating hormone, CRP: C-reactive protein, WBC: White blood cell, FG: Fasting glucose, FMD: Flow-mediated dilation

DBP (79±11 vs. 82±12 mmHg; p=0.04), TG [108 (77-132) vs. 139 (95-183); p<0.001], FBG (87±9 vs. 97±13 mg/dL; p<0.001), 2-h blood glucose (102±26 vs. 130±37 mg/dL; p=0.001) and uric acid levels (4.6±1.0 vs. 5.3±1.3 mg/dL; p<0.001) were significantly lower in MHO than in IRO. The presence of the MS (64% vs. 35%) and the number of the MS criteria were significantly higher in IRO group than in MHO group (p<0.001 for both). CRP [5.2 (3.4-9.1) vs. 6.3 (3.4-9.9) mg/L; p=0.62] and WBC (7.5±1.5 vs. 7.9±2.0; p=0.17) were similar in both groups. The presence of ED was similar in both groups (80% vs. 71%; p=0.25).

The predictors of the percent of FMD change assessed by logistic regression analysis in all participants are given in Table 4. The percent FMD change (<4.5%) was considered as ED (19). None of the MS components (SBP, DBP, FBG, TG, HDL or WC) or CRP was associated with the percent FMD change after adjustment for age, gender and current tobacco use.

Table 2. Comparison of anthropometric, main metabolic parameters and presence of endothelial dysfunction (Δ flow-mediated dilation <4.5%) of the study subjects grouped according to body mass index

	BMI: 30-34.9 (n=77)	BMI: 35-39.9 (n=74)	BMI≥40 (n=80)	p
Age (year)	39±11	41±10	41±12	0.48
Gender (F%)	84	84	81	0.85
Current smoker (%)	17	16	21	0.69
BMI (kg/m^2)	33±1.4	37±1.5	43±3.0	<0.001
WC (cm)	95±9	103±10	113±10	<0.001
SBP (mmHg)	120±16	130±22	128±18	0.004
DBP (mmHg)	78±10	82±13	82±11	0.02
ED (%) (FMD<4.5%)	78	74	75	0.84
TC (mg/dL)	197±39	198±36	202±38	0.64
HDL-C (mg/dL)	47±9	48±10	47±9	0.77
LDL-C (mg/dL)	124±33	125±31	127±31	0.79
TG (mg/dL)	113 (90-152)	111 (87-139)	135 (85-175)	0.25
FG (mg/dL)	92±11	90±10	93±12	0.19
2-h glucose (mg/dL)	109±30	112±31	117±38	0.39
FI (μU/mL)	11 (8-14)	10 (8-16)	13 (10-16)	0.04
HOMA-IR	2.3 (1.6-3.2)	2.3 (1.6-3.8)	2.8 (2.0-3.9)	0.04
ISI	4.2 (2.9-5.6)	4.7 (2.5-6.2)	3.1 (2.1-5.1)	0.005
MS (%)	39	43	65	0.002
MS comp (n)	2.1±0.9	2.3±1.0	2.7±0.9	0.003
Uric acid (mg/dL)	4.6±1.0	4.9±1.2	5.1±1.1	0.007
Crea (mg/dL)	0.67±0.16	0.69±0.33	0.65±0.15	0.61
25(OH)D3 (ng/mL)	10 (7-15)	11 (7-17)	8 (6-12)	0.07
TSH (μIU/mL)	1.8 (1.2-2.7)	2.0 (1.3-2.8)	1.8 (1.2-3.3)	0.66
CRP (mg/L)	4.9 (3.4-7.2)	6.7 (3.4-9.9)	7.3 (4.6-10.6)	0.008
WBC (x10^3/mm^3)	7.6±1.7	7.5±1.6	8.0±1.8	0.18

BMI: Body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure ED: Endothelial dysfunction, TC: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoproein cholesterol, TG: Triglyceride, FI: Fasting insulin, HOMA-IR: Homeostasis model assessment of IR, ISI: Insulin sensitivity index, 25(OH)D3: 25-hydroxyvitamin D3, TSH: Thyroid-stimulating hormone, CRP: C-reactive protein, WBC: White blood cell, MS: Metabolic syndrome, FG: Fasting glucose, FMD: Flow-mediated dilation

Discussion

Obesity is generally accompanied by unfavorable metabolic parameters, such as impaired glucose metabolism, poor lipid profiles and elevated blood pressure, however, not every obese patient has these unfavorable metabolic parameters. Although the definition, significance and prognosis of MHO has not yet been clearly determined, our study results show that the incidence of ED was similar in both phenotypes of obesity in which ages and BMIs were similar.

Table 3. Comparison of the anthropometric, main metabolic parameters and presence of endothelial dysfunction (Δ flow-mediated dilation <4.5%) in insulin-resistant obesity and metabolically healthy obesity subjects

	MHO subjects (n=77)	IRO subjects (n=77)	p
ISIa	6.60 (5.11-16.04)	2.25 (0.95-2.96)	
Age (year)	39±9	40±10	0.59
Gender (F%)	89	81	0.49
Currentsmoker (%)	13	26	0.04
BMI (kg/m^2)	38±5	39±5	0.09
WC (cm)	101±11	106±13	0.01
SBP (mmHg)	123±19	127±17	0.26
DBP (mmHg)	79±11	82±12	0.04
ED (%) (Δ FMD<4.5%)	80	71	0.25
TC (mg/dL)	194±37	205±37	0.09
HDL-C (mg/dL)	48±8	46±9	0.12
LDL-C (mg/dL)	123±32	130±32	0.19
TG (mg/dL)	108 (77-132)	139 (95-183)	<0.001
FG (mg/dL)	87±9	97±13	<0.001
2-h glucose(mg/dL)	102±26	130±37	<0.001
FI (μU/mL)	7 (6-9)	18 (14-22)	<0.001
HOMA-IR	1.5 (1.3-1.9)	4.1 (3.4-5.1)	0.02
MS (%)	35	64	<0.001
MS comp (n)	2.0±0.9	2.7±0.9	<0.001
Uric acid (mg/dL)	4.6±1.0	5.3±1.3	<0.001
Crea (mg/dL)	0.64±0.12	0.67±0.15	0.13
25(OH)D3 (ng/mL)	8 (6-13)	10 (7-15)	0.15
TSH (μIU/mL)	1.9 (1.2-2.8)	1.9 (1.3-3.1)	0.53
CRP (mg/L)	5.2 (3.4-9.1)	6.3 (3.4-9.9)	0.62
WBC (x10^3/mm^3)	7.5±1.5	7.9±2.0	0.17

BMI: Body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure ED: Endothelial dysfunction, TC: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoproein cholesterol, TG: Triglyceride, FI: Fasting insulin, HOMA-IR: Homeostasis model assessment of IR, ISI: Insulin sensitivity index, 25(OH)D3: 25-hydroxyvitamin D3, TSH: Thyroid-stimulating hormone, CRP: C-reactive protein, WBC: White blood cell, MS: Metabolic syndrome, FG: Fasting glucose, FMD: Flow-mediated dilation, IRO: Insulin-resistant obesity, MHO: Metabolically healthy obesity

Determining ED is important because it is the early event which subsequently leads to atherosclerosis, and thus, it predicts which patients are at cardiovascular risk. It is known that FMD is a reliable, non-invasive and widely used method for determining ED (18,20,21). In the literature, cross-sectional studies evaluating subclinical atherosclerosis markers in metabolically healthy defined obese patients are lacking. There are only two studies with a few participants that assessed endothelial function using FMD in MHO subjects. In the first one, the study included 24 metabolically healthy obese subjects and the results were compared with normal weight healthy counterparts (22). In that study, FMD was significantly lower in the healthy obese subjects. In the second study, 65 morbidly obese subjects were included in the study, and MHO was defined as having HOMA-IR <3.5 (23). In that study, FMD was lower in obesity with IR when compared with obesity without IR. There are other studies which used other markers of endothelial function. In a study including 20 non-insulin resistant obese and 32 insulin-resistant obese patients, endothelial function was assessed by measuring serum levels of interleukin-6 and TNF-α. Endothelium-dependent relaxation in response to bradykinin in mesenteric microvessels was also assessed by wire myography (24). In that study, ED was observed only when IR accompanied obesity. In another cross-sectional study including 475 women (25), common carotid artery intima media thickness (CCA-IMT), aortic pulse wave velocity (aPWV) and coronary (CAC) and aortic calcification (AC) were evaluated in healthy normal weight, metabolically healthy overweight/obese and at risk overweight/obese individuals. The mean CCA-IMT and aPWV were lowest in the normal weight group, followed by the benign overweight/obese and at risk overweight/obese groups. Similar results were found for the frequency of women with increased CAC and AC (25).

In a study with 88 postmenopausal women, MHO subjects (determined according to insulin sensitivity) had significantly lower CRP levels when compared with insulin-resistant peers with similar BMI (26). In another study with 5519 participants, CRP levels of the MHO group were between those of metabolically healthy normal weight and MHO subjects (27). Conflicting results from our study indicated that inflammatory markers (CRP and WBC) were not different in MHO and IRO subjects, although CRP correlated to a much greater extent with BMI than with IR status. Moreover, in our study, MS components (SBP, DBP, FBG, TG, HDL or WC) or CRP did not predict the FMD in any of the groups. Some undetermined factors other than the well-known obesity-related risk factors could be responsible for this observation.

Table 4. Predictors of percent Δ flow-mediated dilation in all study subjects

	95% Confidence interval	p*
SBP (mmHg)	1.00 (0.98-1.03)	0.42
DBP (mmHg)	0.99 (0.95-1.02)	0.70
FG (mg/dL)	0.99 (0.96-1.02)	0.61
TG (mg/dL)	1.00 (0.99-1.00)	0.12
HDL-C (mg/dL)	1.00 (0.96-1.04)	0.85
WC (cm)	0.98 (0.95-1.01)	0.38
CRP (mg/L)	0.94 (0.87-1.01)	0.11

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FG: Fasting glucose, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, WC: White blood cell, CRP: C-reactive protein
P* value achieved after adjusment for age, gender and smoking status
Δ flow-mediated dilation <4.5% was considered as endothelial dysfunction

There are studies with conflicting results in which long-term cardiovascular results of MHO were examined. Several prospective studies suggest that MHO individuals are not at increased risk of incident CVD compared with normal weight individuals (28,29,30). However, in a study with 7122 participants (69.7% men) with a median follow-up of 17.4 years, the risk of CVD was increased in both obesity phenotypes compared with healthy normal weight individuals, but the risk was higher in individuals with MHO (hazard ratio, 1.99 vs. 2.49, respectively) (31).

Obesity is associated with IR and MS, however, the presence of abdominal obesity appears to be more extensively correlated with metabolic risk factors than elevated BMI (32,33,34). In our study, the group defined as MHO had significantly lower WC and lower incidence of MS compared with that defined as IRO, although both groups had similar BMI. Thus, while defining MHO, we can use the term, more insulin sensitive obesity. In the light of current and previous studies, we conclude that MHO subjects display a group with an intermediate risk for CVD between metabolically healthy normal weight and metabolically unhealthy obese subjects. Interventions should aim at lowering both weight (change in eating patterns, increasing physical activity and lowering the amount of sedentary time) and metabolic risk factors.

The strength of this study is that it is the largest study with 231 obese participants in which preclinical atherosclerosis examined by FMD in MHO and IRO subjects with no known CVD or diabetes were compared. The major limitation of this study is its cross-sectional design. In addition, majority of the subjects were women, although the men were almost equally distributed among IRO and MHO groups. The study included women aged 18-65 years, but menopausal status was not recorded.

Conclusion

The incidence of ED, assessed by FMD, was similar in both MHO and IRO subjects. Clinicians should be cautious against using terms obesity with metabolically healthy phenotype is safe. Whether there is an increased risk of cardiovascular events and death in MHO remains to be cleared and further studies are needed.

Ethics

Ethics Committee Approval: Kartal Dr. Lütfi Kırdar Training and Research Hospital Ethics Committee approved the study protocol, and informed consent was obtained from all subjects, Informed Consent: Consent was filled out by all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Şule Temizkan, Ayşenur Özderya, Kadriye Aydın, Concept: Şule Temizkan, Design: Şule Temizkan, Data Collection or Processing: Şule Temizkan, Ayşenur Özderya, Şevin Demir, Hilal Toplu Öztürk, Kadriye Aydın, Analysis or Interpretation: Şule Temizkan, Mehmet Sargın, Literature Search: Şule Temizkan, Şevin Demir, Hilal Toplu Öztürk, Writing: Şule Temizkan.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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