Introduction

The worldwide prevalence of obesity has more than doubled and 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

Endothelial Function in Distinct Phenotypes of Obesity

Farklı Obezite Fenotiplerinde Endotelyal Fonksiyon

Şule Temizkan, Ayşenur Özdenya, Şevin Demir*, Hilal Toplu Öztürk*, Mehmet Sargin*, Kadriye Aydın
Kartal Dr. Lütfi Kırdar Training and Research Hospital, Clinic of Endocrinology and Metabolic Diseases, İstanbul, Turkey
*Kartal Dr. Lütfi Kırdar Training and Research Hospital, Clinic of Family Medicine, İstanbul, Turkey

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/m²; 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 endotelyal fonksiyonlarını (ED) değerlendirmeyi amaçladık.

**Gereç ve Yöntem:** Bu kesitsel çalışmaya 231 obez birey (%83 kadın) alınmıştır. Katılımcıların brakial arterindeki Doppler ultrasonografi ile akım aracılı genişleme değerlendirildi ve tüm katılımcılar 75 gram oral glukoz tolerans testi uygulandı. Katılımcıların 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 akım aracılı dilatasyon <%4,5 olarak tanımlandı.

**Bulgular:** Metabolik olarak sağlıklı obez grup ve insülin direnci olan obez grupların benzer yaş (39±9 vs. 40±10 yıld; p=0.59) ve vücut kitle indeksi (38±5 vs. 39±5 kg/m²; p=0.09) sahiptiler. Bel çevresi (101±11 vs. 106±13 cm; p=0.01), açılık kan şekerleri (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 grubundan daha düşük bir düzeyde bulunmuştur. ED görülme sıklığı her iki grupta benzerdir (%80 vs. %71; p=0.25, sırasıyla).

**Tartışma:** ED görülme sıklığı metabolik olarak sağlıklı obezlerde insülin direnci olan obez bireylerde insülin direnci olan obez bireylerdeki gibidir. Çalışmamızda metabolik olarak sağlıklı obezlerde insülin direnci olan obez grubundaki obez grubundaki obez grubunun 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
Obesity and Endothelial Function

Temizkan et al.

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 Kirdar Training and Research Hospital in Istanbul 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 Kirdar 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/m²) 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 lipoprotein 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) x fasting plasma glucose (mg/dL)] / 405 and ISI = [10,000/square root of (fasting glucose x fasting insulin)] x (mean glucose x 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 ≥ 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 measured by the enzymatic method (Abbott Diagnostics, USA).
calculated using Friedewald formula \(\text{LDL-C} = \text{TC} - \text{TG} / 5 + \text{HDL-C}\). 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 Apio 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 or median (25% and 75% interquartiles) for non-normally distributed variables or median (25% and 75% interquartiles) for non-normally distributed variables. The one-way ANOVA for normally distributed variables, the Mann-Whitney U test for non-normally distributed variables, and the Kruskal-Wallis test for non-normally distributed variables were the 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/m² and male: 38±5 kg/m²) 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), BMI were similar in IRO and MHO groups. 25(OH)D and TSH levels were similar in both BMI groups, but MS was positive in 39% of patients in group 1, 43% in group 2 and 65% in group 3.

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 upper tertile (n=77). Logistic regression analysis was performed to determine independent predictors of the percent of ΔFMD in all participants. 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), BMI were similar in IRO and MHO groups. 25(OH)D and TSH levels were similar in both 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), BMI were similar in IRO and MHO groups. 25(OH)D and TSH levels were similar in both BMI groups, but MS was positive in 39% of patients in group 1, 43% in group 2 and 65% in group 3.
DBP (79±11 vs. 82±12 mmHg; p=0.04), TG (108 [77-132] vs. 139 [95-183]; p<0.001), Fasting 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 compared to MHO group (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, Fasting glucose, TG, HDL or WC) or CRP was associated with the percent FMD change after adjustment for age, gender and current tobacco use.

### 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 BMIa were similar.

### 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MHO (n=77)</th>
<th>MHO (n=74)</th>
<th>MHO (n=80)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>39±11</td>
<td>41±10</td>
<td>41±12</td>
<td>0.48</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>84</td>
<td>84</td>
<td>81</td>
<td>0.85</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>17</td>
<td>16</td>
<td>21</td>
<td>0.69</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27±2</td>
<td>32±2</td>
<td>32±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>95±9</td>
<td>103±10</td>
<td>113±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120±16</td>
<td>130±22</td>
<td>128±18</td>
<td>0.004</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78±10</td>
<td>82±13</td>
<td>82±11</td>
<td>0.02</td>
</tr>
<tr>
<td>ED (%) (Δ FMD&lt;4.5%)</td>
<td>78</td>
<td>74</td>
<td>75</td>
<td>0.84</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>197±39</td>
<td>198±36</td>
<td>202±38</td>
<td>0.64</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>47±9</td>
<td>48±10</td>
<td>47±9</td>
<td>0.77</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>124±33</td>
<td>125±31</td>
<td>127±31</td>
<td>0.79</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>113 (90-152)</td>
<td>111 (87-139)</td>
<td>135 (85-175)</td>
<td>0.25</td>
</tr>
<tr>
<td>FG (mg/dL)</td>
<td>92±11</td>
<td>90±10</td>
<td>93±12</td>
<td>0.19</td>
</tr>
<tr>
<td>2-h glucose (mg/dL)</td>
<td>109±30</td>
<td>112±31</td>
<td>117±38</td>
<td>0.39</td>
</tr>
<tr>
<td>FI (μU/mL)</td>
<td>11 (18-14)</td>
<td>10 (18-16)</td>
<td>13 (10-16)</td>
<td>0.04</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.3 (1.3-2.0)</td>
<td>2.1 (1.3-3.8)</td>
<td>2.8 (2.0-3.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>ISI</td>
<td>4.2 (2.9-5.6)</td>
<td>4.7 (2.5-6.2)</td>
<td>3.1 (2.1-5.1)</td>
<td>0.005</td>
</tr>
<tr>
<td>MS (%)</td>
<td>39</td>
<td>43</td>
<td>65</td>
<td>0.002</td>
</tr>
<tr>
<td>MS comp (n)</td>
<td>2.1±0.9</td>
<td>2.3±1.0</td>
<td>2.7±0.9</td>
<td>0.003</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.6±1.0</td>
<td>4.9±1.2</td>
<td>5.1±1.1</td>
<td>0.007</td>
</tr>
<tr>
<td>Crea (mg/dL)</td>
<td>0.67±0.16</td>
<td>0.69±0.33</td>
<td>0.65±0.15</td>
<td>0.61</td>
</tr>
<tr>
<td>25(OH)D3 (ng/mL)</td>
<td>10 (7-15)</td>
<td>11 (7-17)</td>
<td>8 (6-12)</td>
<td>0.04</td>
</tr>
<tr>
<td>TSH (μU/mL)</td>
<td>1.8 (1.2-2.7)</td>
<td>2.0 (1.3-2.8)</td>
<td>1.8 (1.2-3.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.9 (3.4-7.2)</td>
<td>6.7 (3.4-9.9)</td>
<td>7.3 (4.6-10.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>WBC (x10³/mm³)</td>
<td>7.6±1.7</td>
<td>7.5±1.6</td>
<td>8.0±1.8</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Determining ED is important because it is the early event which subsequently leads to atherosclerosis, and thus, if 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.

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 (67.7% 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 Kirdar 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


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

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

References