Leptin is Associated with Endothelial Dysfunction in Healthy Obese Premenopausal Women

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ABSTRACT

Background and Objectives: Previous studies have demonstrated that adipokines can have positive and/or negative effects on vascular function. In this study, we attempted to characterize the association of adipokines with endothelium-dependent vasodilation in healthy premenopausal women. Subjects and Methods: Noninvasive pulse wave analysis coupled with provocative pharmacological testing with salbutamol was used to measure endothelium-dependent vasodilation in 60 healthy premenopausal women [37 obese women; body mass index (BMI) ≥ 25 kg/m², 23 age-matched non-obese women; BMI < 25 kg/m²]. The lipid profile, fasting insulin, glucose, and C-reactive protein (CRP) concentrations in each patient were assessed via standard laboratory techniques, and plasma concentrations of various adipokines, such as adiponectin, leptin, resistin and TNF-α, were measured via enzyme immunoassays.

Results: In the obese group, higher leptin concentrations were significantly associated with impairments in endothelium-dependent vasodilation (r = -0.371, p = 0.005). This association remained significant, even after adjustment for other risk factors (β = -0.39, p = 0.006). However, we determined that there was no significant correlation between endothelium-dependent vasodilation and these variables in the obese group and the control group.

Conclusion: Increased plasma concentration of leptin was associated with impairment in endothelial function in obese premenopausal women, regardless of the metabolic and inflammatory disturbances associated with obesity.

KEY WORDS: Endothelium; Vascular; Leptin; Obesity.
et al. demonstrated that salbutamol, a β2-agonist, can induce vasodilation via the L-arginine-NO pathway. Chowienczyk et al. demonstrated that salbutamol, a β2-agonist, reduces peripheral pressure wave reflection by activating the L-arginine-NO pathway. Arterial waveform changes occurring after salbutamol treatment have been shown to provide a noninvasive method for the measurement of "global" arterial endothelial function, and these changes have also been associated with invasive methods involving intra-arterial acetylcholine infusion, as evidenced by applications of the standard method of endothelial function measurement.

In the present study, we attempted to characterize the association between adipokines and endothelial dysfunction, as measured by pulse wave analysis, as well as its relationship to the inflammatory and metabolic disturbances that are associated with obesity. Thus, the study of this association may prove to be of great importance, as it may provide new insights into both prevention and early diagnostic strategies.

Subjects and Methods

Subjects

The subjects of this study were all premenopausal women between 20 and 45 years of age. Obese subjects with body mass indices (BMI) ≥25 kg/m² were selected from among the individuals who visited the university hospital’s obesity clinic. The set of criteria used to define obesity in Asian adults was adapted from the guidelines provided by ‘The Asia-Pacific perspective: Redefining Obesity and its Treatment’—a joint enterprise of the Regional Office for the Western Pacific of the World Health Organization, the International Association for the Study of Obesity and the International Obesity Task Force’. All subjects were free of any acute or chronic disease conditions, such as hypertension, dyslipidemia (low-density lipoprotein cholesterol ≥160 mg/dL, high-density lipoprotein cholesterol <40 mg/dL or triglyceride ≥200 mg/dL), type II diabetes, coronary heart disease, stroke, thyroid disease, and depression. Current smokers were also excluded from the study. Age-matched premenopausal volunteers with BMI values of less than 24 kg/m² were employed as control subjects. All of the subjects in this study provided informed consent, and the study protocols were pre-approved by the Institutional Review Board at Ewha Womans University Mokdong Hospital.

Biochemical measurements

All analyses were conducted on blood samples that were drawn after at least 12 hours of overnight fasting and stored at -70℃ until the assay. The blood samples were obtained only during the follicular phase of the menstruation cycle. The glucose oxidase method was used to measure the plasma glucose concentrations. The immunoreactive insulin concentrations were measured in plasma via radioimmunoassay, using a modification of the double-antibody method. Homeostasis Model Assessment (HOMA) scores were calculated in order to assess insulin resistance. Estradiol levels were determined via microparticle enzyme immunoassay (Axisym, Abbott, USA). Hs-CRP levels were determined with an ultrasensitive CRP test. The results had coefficients of variation (CV) of less than 5% (N Latex CRP: Dade Behring Co Ltd). Plasma triglyceride and total cholesterol levels were measured enzymatically. High-density lipoprotein (HDL) cholesterol was precipitated using dextran-sulphate and measured enzymatically. Low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedewald equation: LDL cholesterol = total cholesterol - [HDL cholesterol + (triglycerides/5)]. Plasma adiponectin levels were determined using a commercially available ELISA kit (AdipoGen Inc, Seoul, Korea) with a detection limit of 0.1 ng/mL (inter-assay and intra-assay CV values...

\[ \text{Augmentation index (AIx)} = \frac{b - a}{a + b} \times 100\% \]

**Fig. 1.** The augmentation index is a measure of the effects of wave reflection on the second systolic peak and is thus a measure of the additional load to which the left ventricle is subjected as a result of wave reflection. The augmentation index is calculated as the increment in pressure from the first shoulder in the ascending aortic pressure wave (a) to the peak of this wave (b), and it is expressed as a percentage of the peak ascending aortic pressure wave. AIx: augmentation index.
were 3.5% and 4.4%, respectively). Resistin levels were determined with the use of a commercially available ELISA kit (AdipoGen Inc, Seoul, Korea). The sensitivity of the assay was 0.1 ng/mL, the standard range was 0.125–8 ng/mL, and the intra-assay CV values and inter-assay CV values were 3.7% and 5.6%, respectively. Plasma leptin levels were measured using an ELISA kit (BioSource International Inc. USA, Camarillo California). The sensitivity of the assay was <3.5 pg/mL, and the inter-assay CV values and intra-assay CV values were 4.6% and 3.6%, respectively. TNF-α levels were measured with an ELISA kit (R & D Systems and Phoenix Pharmaceuticals, USA). The sensitivity of the assay was 3.0 pg/mL, the standard range was 0.1 ng/mL, the standard range was 0.125–8 ng/mL, and the intra-assay CV values and inter-assay CV values were 3.7% and 5.6%, respectively. The coefficient of variance (CV) for the pulse wave analysis was determined with the use of a commercially available ELISA kit (AdipoGen Inc, Seoul, Korea). The sensitivity of the assay was 3.0 pg/mL, the standard range was 0.1 ng/mL, the standard range was 0.125–8 ng/mL, and the intra-assay CV values and inter-assay CV values were 3.7% and 5.6%, respectively.

Endothelium-dependent vasodilation

Pulse wave analysis was conducted with a provocative pharmacological test in order to assess vascular endothelial dysfunction.

Pulse wave analysis (SphygmoCor, Medical ECONET Co., Australia)

The subjects were studied in a quiet temperature-controlled laboratory (26 ± 1°C) after 20 minutes of lying supine. Aix was determined by application of tonometry from the left radial artery using a high-fidelity micromanometer (SPC-301; Millar Instrument, TX, USA). Aortic pressure was assessed according to transfer function, as in previous studies. The augmentation index (Aix), which refers to the stiffness of the arterial wall, was calculated using the appropriate computer software.

Measurement of AIX via provocative pharmacological tests

Aix was determined after the administration of nitroglycerin, which induces vasodilation independently of the vascular endothelial cells, and salbutamol, which induces vasodilation via the vascular endothelial cells, respectively.

ΔAix(Nitroglycerin, %)

The difference in Aix before and after the administration of 0.6 mg nitroglycerin was calculated, and the maximum difference was depicted as ΔAix(Nitroglycerin, %).

ΔAix(Salbutamol, %)

Twenty-five minutes after the peak action time of nitroglycerin, 2.5 mg of nebulized salbutamol (ventolin nebul, GlaxoSmithKline) were administered by supervised inhalation. The difference in Aix before and after the administration of this drug was calculated and the maximum difference was depicted as ΔAix(Salbutamol, %).

The pharmacological test in order to assess vascular endothelial dysfunction was performed with the SPSS 10 software. All statistical calculations were performed with the SPSS 10 software.

Baseline characteristics

The anthropometric, demographic, and metabolic characteristics of the subjects are shown in Table 1. The plasma leptin levels were significantly higher in the obese group than in the controls. The plasma adiponectin levels were significantly lower in the obese group, but the resistin and TNF-α levels did not differ significantly between the two groups (Table 1).

Pulse wave analysis

There were no significant differences with regard to changes in baseline heart rate, baseline Aix, and baseline MAP. Our results indicated no significant change in heart rate or MAP following the administration of either NTG or salbutamol. The Aix after NTG was also unchanged. On the other hand, the ΔAix(Salbuta-
Table 1. Baseline characteristics of the obese and the non-obese women

<table>
<thead>
<tr>
<th></th>
<th>Obese (n=57)</th>
<th>Controls (n=23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>34 ± 7</td>
<td>31 ± 7</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.1 ± 3.6</td>
<td>20.5 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>199.6 ± 23.9</td>
<td>179.6 ± 27.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>108.6 ± 45.7</td>
<td>76.7 ± 22.5</td>
<td>0.005</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>98.1 ± 19.1</td>
<td>81.2 ± 16.6</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>54.9 ± 7.9</td>
<td>60.4 ± 4.9</td>
<td>0.007</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>97.8 ± 8.3</td>
<td>97.0 ± 7.7</td>
<td>0.73</td>
</tr>
<tr>
<td>Fasting insulin (μU/mL)*</td>
<td>15.0 ± 8.6</td>
<td>4.9 ± 5.9</td>
<td>0.023†</td>
</tr>
<tr>
<td>HOMA score*</td>
<td>3.8 ± 5.2</td>
<td>1.2 ± 1.5</td>
<td>0.032‡</td>
</tr>
<tr>
<td>NEFA (μEq/L)</td>
<td>507.3 ± 242.3</td>
<td>398.9 ± 235.5</td>
<td>0.11</td>
</tr>
<tr>
<td>hs-CRP (mg/L)*</td>
<td>0.24 ± 0.31</td>
<td>0.03 ± 0.02</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>227.2 ± 88.4</td>
<td>255.4 ± 82.5</td>
<td>0.072</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>31.28 ± 14.0</td>
<td>16.44 ± 9.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>3.24 ± 1.08</td>
<td>4.90 ± 2.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resistin (ng/mL)</td>
<td>3.30 ± 1.4</td>
<td>3.63 ± 1.490</td>
<td>0.39</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.76 ± 1.4</td>
<td>1.64 ± 0.4</td>
<td>0.45</td>
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</tbody>
</table>

Values are mean ± SD. BMI: body mass index, hs-CRP: high-sensitivity C-reactive protein, NEFA: non-esterified free fatty acid, LDL: low-density lipoprotein, HDL: high-density lipoprotein, HOMA: homeostasis model assessment, TNF-α: tumor necrosis factor-α, †: hs-CRP, fasting insulin and HOMA score were presented as median ± interquartile differences. ‡: hs-CRP fasting insulin, and HOMA score are logarithmically transformed before analysis.

Table 2. Results of pulse wave analysis in obese women and non-obese women

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>117 ± 10</td>
<td>112 ± 11</td>
<td>0.298</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>73 ± 6</td>
<td>68 ± 8</td>
<td>0.554</td>
</tr>
<tr>
<td>Baseline HR (beat/min)</td>
<td>72.1 ± 7.4</td>
<td>71.4 ± 11.4</td>
<td>0.887</td>
</tr>
<tr>
<td>Baseline Alx (%)</td>
<td>22.9 ± 8.8</td>
<td>19.9 ± 10.3</td>
<td>0.292</td>
</tr>
<tr>
<td>Baseline MAP (mmHg)</td>
<td>87.8 ± 1.2</td>
<td>84.8 ± 1.5</td>
<td>0.134</td>
</tr>
<tr>
<td>Change in HR (NTG, beat/min)</td>
<td>14.9 ± 9.0</td>
<td>12.8 ± 7.6</td>
<td>0.364</td>
</tr>
<tr>
<td>Change in HR (salbutamol, beat/min)</td>
<td>5.5 ± 4.8</td>
<td>4.4 ± 6.6</td>
<td>0.407</td>
</tr>
<tr>
<td>Change in MAP (NTG, beat/min)</td>
<td>1.8 ± 0.4</td>
<td>1.5 ± 0.2</td>
<td>0.312</td>
</tr>
<tr>
<td>Change in MAP (salbutamol, beat/min)</td>
<td>2.0 ± 0.3</td>
<td>3.5 ± 0.5</td>
<td>0.195</td>
</tr>
<tr>
<td>Change in Alx (NTG, beat/min)</td>
<td>30.9 ± 9.7</td>
<td>30.7 ± 10.1</td>
<td>0.917</td>
</tr>
<tr>
<td>Change in Alx (salbutamol, beat/min)</td>
<td>11.3 ± 6.7</td>
<td>18.2 ± 6.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD. SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, Alx: augmentation index, NTG: nitroglycerin, MAP: mean arterial pressure.

m, %) values were found to be much lower in the obese group than in the control group (11.3 ± 6.7% vs. 18.2 ± 6.8%, p < 0.001), which supports the notion that endothelium-dependent vasodilatation was impaired in the obese group (Table 2).

Relationships between endothelium-dependent vasodilation (Alx(Salbutamol, %)) and metabolic, variables, and adipokines

There was a negative correlation between endothelium-dependent vasodilation and plasma leptin concentration in the obese group. However, the concentration of adiponectin was positively correlated with endothelium-dependent vasodilation (Fig. 2). Our univariate regression analyses, which were adjusted according to potential confounding factors including age, heart rate, baseline and change in MAP revealed that the Alx(Salbutamol, %) value could be negatively correlated with leptin, total cholesterol, LDL-cholesterol, BMI, hs-CRP, and HOMA score. There was a positive correlation between Alx(Salbutamol, %) value and adiponectin (Table 3). The levels of resistin were not significantly correlated with Alx(Salbutamol, %).

In the control group, there was no significant correlation between the Alx(Salbutamol, %) value and these metabolic variables, such as the level of leptin, adipokine, hs-CRP and other metabolic variables (Table 3).

We fitted a stepwise multiple regression model with Alx(Salbutamol, %), which was used as the dependent variable. We used age, BMI, total cholesterol, LDL-cholesterol, hs-CRP, HOMA score, fasting insulin, adiponectin levels, and leptin levels as potential explanatory variables. The final model accounted for 53% of the variability in the Alx(Salbutamol, %). Endothelium-dependent vasodilation was significantly correlated with the levels of leptin (β = -0.39, p = 0.006) and hs-CRP (β = -0.36, p = 0.015). Leptin was the only adipokine to significantly and independently associate with endothelium-dependent vasodilation (Table 4).

Discussion

The novel finding of the present study is that leptin is independently associated with endothelial dysfunction in obese premenopausal women without any conventional cardiovascular risk factors. This association between leptin and endothelial dysfunction was significant, even after adjustments were made for the metabolic and inflammatory disturbances typically associated with obesity. This finding, together with the evidence gleaned from previously-constructed experimental models and prospective studies that have demonstrated the effects of leptin on clinical outcomes, strongly suggests that leptin may be one of the molecular links by which obesity is linked to the early stages of atherosclerosis.

The effects of adipokines on vascular function were assessed in patients with diabetes, hypertension, and coronary artery disease. However, the effects of obesity and adipokines on vascular function in patients without these conditions need further investigation.
diseases have not been assessed. We opted to focus on premenopausal women who showed few, if any, of the potential confounding factors that are frequently detected in older, diseased populations with hypertension, dyslipidemia, and insulin resistance. Furthermore, we only studied women in order that we might be able to completely dismiss the possibility of confounding effects due to sex-based differences. In addition, the blood tests were conducted only during the follicular phase of the menstrual cycle in order to preclude the possibility of estrogen-related effects on inflammatory markers and hs-CRP.28

Previous studies proposed several mechanisms by which leptin might mediate arterial disease.20-22 Although leptin receptors are predominantly involved in the hypothalamic control of body weight, they are widely distributed throughout endothelial cells,20 within other arterial subpopulations,21 and in atherosclerotic plaque.20,22 Leptin may stimulate the proliferation and migration of vascular smooth muscle cells via these receptors,21 whereas prolonged leptin treatment has been shown to increase the rate at which vascular calcification occurs.23 Leptin has also been shown to stimulate the generation of proinflammatory cytokines from cultured monocytes and to induce oxidative stress in endothelial cells, which might contribute to vascular pathology.23 Furthermore, the notion that leptin exerts a direct influence on vascular health is supported by the existence of the ob/ob mouse, which lacks leptin, and consequently becomes hyperphagic and grossly obese, but nevertheless appears to be resistant to atherosclerosis.25 The risk of atherosclerosis in heterozygotes lies between that of ob/ob homozygotes and that of control animals, which is suggestive of a dose-response relationship between leptin levels and the atherosclerotic process.25 Obese humans exhibit increased leptin production per unit of fat mass, and therefore tend to manifest disproportionately elevated leptin concentrations.20

Unlike many other adipokines, including TNF-α, leptin, and resistin, the levels of all of which are known to increase with adiposity,27 the concentrations of circulating adiponectin tend to be reduced in obese indivi-
Hypoadiponectinemia has been detected in patients suffering from diabetes, hypertension, and CAD, and also appears to be associated, at least to some degree, with increases in the levels of CRP and insulin sensitivity. Adiponectin also appears to affect vascular functions. Low plasma adiponectin levels are associated with impairments in endothelium-dependent vasodilation, and this association appears to be independent of the existence of diabetes mellitus in the individual. In this study, we determined that adiponectin levels tended to be significantly lower in the obese group than in the control group, and they were negatively correlated with both insulin resistance and hs-CRP. Although the levels of adiponectin were correlated with endothelium-dependent vasodilation, our univariate regression analysis indicated that the adiponectin levels did not constitute independent predictors of endothelial dysfunction, according to the results of our multivariate regression analysis. However, the number of subjects in this study was small, and it would have been preferable to investigate the effect of adiponectin on endothelial function with a larger number of subjects.

The results of previous studies revealed that CRP not only reflects the inflammatory state of the vascular endothelial cells, but it also leads to a direct reduction in NO synthesis, thereby inducing endothelial dysfunction and cardiovascular disease. In the present study, we confirmed an independent association between the levels of hs-CRP and endothelial dysfunction in premenopausal women with no cardiovascular risk factors.

Potential limitations

The number of subjects in this study was small and the results of our study did not prove a causal association between leptin concentration and vascular dysfunction. However, our results, which indicated that leptin exerts independent effects on vascular health, were supported by recent prospective observations that leptin constitutes an independent risk factor for coronary events. This observation, together with our findings in healthy obese premenopausal women, suggests that the effect of leptin on arterial health is clinically relevant and is not merely epiphenomenon.

Finally, BMI was used to evaluate obesity, but we did not measure the waist-hip ratio or the percent body fat of the subjects. Because the waist-hip ratio is closely associated with parameters related to insulin resistance and because visceral fat is more strongly associated with insulin resistance than subcutaneous fat, measurements of the waist-hip ratio and percent body fat may have been preferable to measurement of BMI.
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