Original Article

Relationship between Changes in Serum Leptin Levels and Blood Pressure after Weight Loss

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Insulin resistance is thought to raise blood pressure. Recently, a significant positive relationship between mean blood pressure and plasma leptin levels, but there have been no reports dealing with the relationship between blood pressure and either insulin resistance or serum leptin levels after weight loss. In the present work, we attempted to clarify the relationship between changes in blood pressure and either the serum leptin level or the insulin level in 102 moderately obese females (mean body mass index (BMI), 29.5 ± 0.5 kg/m²; age, 47.0 ± 0.9) during a 3 month period. No differences in age, fat-mass, homeostasis model assessment (HOMA), the summation of insulin (IRI), plasma renin activity (PRA) or 24 h norepinephrine excretion (24hU-NE) were observed between the hypertensive (HT) group (n = 31) and normotensive (NT) group (n = 71) before weight loss, but the basal serum leptin was significantly higher in the HT (16.8 ± 1.1 ng/ml) than in the NT group (15.2 ± 0.8 ng/ml), after adjusting for abdominal total fat. After a 3 month weight reduction program, the total abdominal fat, serum leptin and IRI significantly decreased in both groups. The systolic blood pressure (SBP)/diastolic blood pressure (DBP) significantly decreased from 144/84 to 130/77 mmHg only in the HT but not in the NT group. The PRA decreased in both groups, while the 24hU-NE significantly decreased only in the HT group. The changes in the leptin level were significantly correlated with the changes in both IRI and HOMA after weight loss in the two groups, respectively. Finally, a statistically significant positive correlation was observed between the changes in the leptin and the changes in the mean blood pressure (MBP) (r = 0.412, p < 0.05) only in the HT group. Multiple regression analysis revealed that the changes in MBP were independently associated with the changes in 24hU-NE and the changes in either IRI or HOMA in all subjects. However, a statistically significant positive correlation was observed between the changes in MBP and the changes in leptin levels even after adjusting for the total abdominal fat, 24hU-NE and either IRI or HOMA (both expressed as a percentage of the baseline value) in a multiple regression analysis only in the HT group. These results suggest that leptin may play a role in the pathophysiology of obese hypertension. (*Hypertens Res 2002; 25: 881–886)

Key Words: leptin, insulin, norepinephrine, blood pressure, obesity

Introduction

Obesity is one of the main risk factors for such diseases as hypertension (1), atherosclerosis (which leads to ischemic heart disease), diabetes, and hyperlipidemia, and in order to cure such diseases it will first be necessary to understand the mechanisms of insulin resistance and thereby prevent obesity.
(2, 3). Previously, we reported that hyperinsulinemia play an important role in the pathogenesis of blood pressure elevation due to obesity not only during weight loss, but also during the weight rebound phenomenon (4).

The obesity gene product leptin (5), the gene which leads to obesity in genetically obese ob/ob mice, is a new hormone secreted from the adipose tissue fat cells identified by the positional cloning method. We know from the activation of the leptin receptors, which mainly appear in the hypothalamus, that leptin has various biological effects, such as feeding inhibition and accelerated energy consumption due to increased sympathetic nerve activity (6–9).

It has already been reported that both the suppression of norepinephrine and improved insulin resistance in hypertension accompanying weight loss in moderately obese people (10). The plasma leptin level in humans has been reported to be closely correlated with both the fat mass and the subcutaneous fat (11–13). Segal et al. reported that insulin resistance was associated with elevated plasma leptin levels independent of body fat mass (14). On the other hand, Mohamed-Ali et al. reported that plasma leptin correlated with insulin concentration, but not with insulin resistance (15). The possible relation of serum leptin level to insulin resistance syndrome remains controversial. A recent study reported that mean blood pressure (MBP) and plasma leptin levels showed a significant positive correlation (16), but the relationship between changes in the leptin level and changes in blood pressure after weight loss have yet to be elucidated.

In this study, we examined the effects of weight loss on blood pressure and serum leptin concentrations, both of which are closely correlated with insulin and insulin-related variables, as well as the correlation between weight loss-induced blood pressure changes and each of insulin level, total abdominal fat and 24-h urinary norepinephrine excretion (24-hU-NE) as an index of sympathetic nervous system activity.

Methods

Subjects and Methods

The subjects in this study were 102 female volunteers (mean age: 47.0 ± 0.9 years) who had attended the Health Improvement Center of Nakamura Gakuen University at some point between 1995 and 2000 in order to lose weight. Subjects whose fasting blood glucose level was over 126 mg/dl were excluded from the present study. None of the subjects in this study were taking any medications, and all were assessed as having healthy lifestyles. All subjects were moderately obese, with an average body mass index (BMI) of 29.5 ± 0.5 kg/m² at the beginning of the weight loss period. Briefly, the dietary program was based upon an energy reduction of 500 kcal/day from the value thought necessary to maintain their weight. The subjects were instructed to follow a diet consisting of 1,400 kcal/day given as 1.5 g/body weight (kg)/day of protein, 30 g fat and 20 g of dietary fiber and if possible, to walk for about 40 min per day. This regimen continued for 3 months. The average of three successive measurement was used as an individual blood pressure and heart rate values. The blood pressure and heart rate were measured the start and 3 months after beginning the weight reduction program, using an automated blood pressure device (BP-8800, Nippon Colin Co. Ltd., Komaki, Japan). Individual blood pressure and heart rate values were taken as the average of three successive measurements made in the morning with subjects in a seated position following an at least 5-min period of quiet rest. All subjects fasted for at least 12 h and were kept in a supine position for 30 min before blood sampling. The plasma glucose and insulin (IRI) were determined before and at 30, 60, 120 min after the oral administration of 75 g of oral glucose (75 g OGTT) at the start and 3 months later. The abdominal fat area was also determined at the beginning and the end of the weight loss period by magnetic resonance imaging (MRI) at the L4-L5 level. Urine samples were collected for 24-h on the day before each blood sampling using 6 eq/l HCl. The subjects were classified into a high-normal and hypertension group (HT group; n = 31; systolic blood pressure (SBP) ≥130 mmHg and/or diastolic blood pressure (DBP) ≥85 mmHg) and a normotensives group (NT group; n = 71; SBP < 130 mmHg and DBP < 85 mmHg) based on JNC-VI (17). Resistance to insulin-mediated glucose disposal was determined by a modification of homeostasis model assessment (HOMA) (18) of 75 g OGTT. The insulin level was determined by ΣIRI (ΣIRI = IRI (0’) + IRI (30’) + IRI (60’) + IRI (120’)). The protocol was approved by the Human Investigation Review Committee of Nakamura Gakuen University. The subjects were fully informed of the program and written informed consent was obtained from all volunteers.

Measurement

MRI was used to measure the cross-sectional areas of adipose tissue (cm²) of the abdominal visceral fat (V), subcutaneous fat (S) and total fat (T = V + S). The areas of V, S and T fat in MRI were determined using a commercially available software package (19). The percent body fat was measured using the Bioelectrical Impedance Measurement method (20). The fat mass was calculated as BW [%] fat (kg). The serum leptin levels were determined at SRL Inc. (Tokyo, Japan) using a radioimmuno assay kit (Lionco Research, St. Charles, USA) as described by Zhongmin et al. (21). The following urinary variables were measured: urinary volume and urinary excretion of Na, K, Ca, Mg, creatinine, aldosterone, epinephrine, and norepinephrine. The plasma insulin was determined by an enzyme immunoassay using a commercial kit (Dinabot Laboratory, Tokyo, Japan). The plasma renin activity (PRA) was measured by a radioimmunoassay. The urinary catecholamine concentrations were measured by HPLC at the Bristol Laboratory in Tokyo. Electrolytes in the urine were measured using atomic absorption spectrophotometry (AA660: Shimadzu Co. Ltd., Tokyo, Japan).
The statistical analysis required a transformation of the data to a near-normal form before testing based on the logarithmic changes in the non-normal distribution. The results are expressed as the mean $\bar{x}$ SEM, and the unpaired Student’s t-test, Mann-Whitney test and regression analysis covariance were used to compare between the means of the two groups. The significance of differences in the findings before and after weight loss were determined using the paired t-test for variables which showed an approximately normal distribution. Changes in the parameters between the start and end of the program were expressed as a percentage of the values at the start of the program. Any correlation between the blood pressure and related variables were tested by Pearson’s correlation coefficient and a multiple regression analysis using the FACOM ANALYST program (Fujitsu, Tokyo, Japan). Levels of $p < 0.05$ were considered to be indicate statistically significance.

**Results**

As shown in Table 1, there were no significant differences in age, heart rate, fat-mass, total abdominal fat, HOMA, $\Sigma$IRI, 24hU-NE, or PRA between the two groups at the beginning of the program. However, the mean BMI was significantly higher in the HT than in the NT group. The serum leptin in the HT group was significantly higher than the NT group after adjusting for either the BMI or abdominal total fat, as closely related to leptin, using a regression analysis covariance ($p < 0.05$).

As shown in Table 1, after the 3-month weight reduction program, the BMI, fat-mass, total abdominal fat and $\Sigma$IRI significantly decreased in both groups, but there was no difference in HOMA in the HT group. Both the SBP and DBP decreased from 144/84 to 130/77 mmHg, and the 24hU-NE significantly decreased only in the HT group, but no such differences were seen in the NT group. No changes

<table>
<thead>
<tr>
<th>Variables</th>
<th>0M</th>
<th>3M</th>
<th>NT</th>
<th>3M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>49.5 $\pm$ 1.5</td>
<td>46.2 $\pm$ 1.2</td>
<td>49.5 $\pm$ 1.5</td>
<td>46.2 $\pm$ 1.2</td>
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<tr>
<td>SBP (mmHg)</td>
<td>143.5 $\pm$ 2.2</td>
<td>130.4 $\pm$ 2.3***</td>
<td>115.8 $\pm$ 1.1‰</td>
<td>113.5 $\pm$ 1.3</td>
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<tr>
<td>DBP (mmHg)</td>
<td>83.7 $\pm$ 1.4</td>
<td>77.3 $\pm$ 1.9**</td>
<td>69.2 $\pm$ 0.9‰</td>
<td>66.9 $\pm$ 0.9</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>113.3 $\pm$ 1.5</td>
<td>104.3 $\pm$ 1.7***</td>
<td>91.8 $\pm$ 0.8‰</td>
<td>89.7 $\pm$ 0.9</td>
</tr>
<tr>
<td>Heart rate (bit/min)</td>
<td>73.2 $\pm$ 2.0</td>
<td>72.4 $\pm$ 2.4</td>
<td>72.1 $\pm$ 1.1</td>
<td>70.6 $\pm$ 1.2</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>29.9 $\pm$ 0.5</td>
<td>28.6 $\pm$ 0.5**</td>
<td>28.3 $\pm$ 0.4</td>
<td>26.9 $\pm$ 0.3**</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>28.1 $\pm$ 0.9</td>
<td>25.6 $\pm$ 0.9***</td>
<td>26.5 $\pm$ 0.6</td>
<td>23.8 $\pm$ 0.6***</td>
</tr>
<tr>
<td>Total abdominal fat (cm$^2$)</td>
<td>364 $\pm$ 11</td>
<td>336 $\pm$ 12***</td>
<td>358 $\pm$ 8.8</td>
<td>312 $\pm$ 8.6***</td>
</tr>
<tr>
<td>Serum leptin (ng/ml)</td>
<td>16.8 $\pm$ 1.1</td>
<td>13.1 $\pm$ 1.3***</td>
<td>15.2 $\pm$ 0.8</td>
<td>11.0 $\pm$ 0.6***</td>
</tr>
<tr>
<td>$\Sigma$IRI (µU/ml)</td>
<td>201.3 $\pm$ 18.1</td>
<td>163.7 $\pm$ 16.3***</td>
<td>202.1 $\pm$ 13.9</td>
<td>160.9 $\pm$ 11.0***</td>
</tr>
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<td>HOMA</td>
<td>2.05 $\pm$ 0.18</td>
<td>1.81 $\pm$ 0.16</td>
<td>2.00 $\pm$ 0.16</td>
<td>1.56 $\pm$ 0.11*</td>
</tr>
<tr>
<td>Urinary NE (µg/day)</td>
<td>140.2 $\pm$ 11.9</td>
<td>118.7 $\pm$ 12.1*</td>
<td>136.9 $\pm$ 16.3</td>
<td>119.7 $\pm$ 9.8</td>
</tr>
<tr>
<td>PRA (ng/ml/h)</td>
<td>0.66 $\pm$ 0.11</td>
<td>0.44 $\pm$ 0.07*</td>
<td>0.81 $\pm$ 0.12</td>
<td>0.63 $\pm$ 0.08*</td>
</tr>
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BP, blood pressure; NE, norepinephrine. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significantly different from the values at baseline. Š $p < 0.05$, Š Š $p < 0.01$, Š Š Š $p < 0.001$: significantly different from the mean values for the HT group.
In the 24-h urinary creatinine level, sodium excretion, or heart rate were seen in either group between the start and end of the weight loss period (data not shown). A statistically significant decrease in serum leptin level due to weight loss was observed after 3 months in both groups (p < 0.01, respectively).

In both groups, there was a significant positive correlation between the log serum leptin level and each of BMI (r = 0.421, p < 0.001), T fat (r = 0.480, p < 0.001), V fat (r = 0.289, p < 0.01), fat-mass (r = 0.493, p < 0.001) and log ΣIRI (r = 0.479, p < 0.001) but no significant correlation was found between log serum leptin levels and blood pressure at the start of the program. However, the relationship between the serum leptin level and BMI, and that between the serum leptin level and fat-mass were not statistically significant after adjusting for total abdominal fat. Furthermore, a significantly positive correlation between not only the log serum leptin and log ΣIRI at the beginning of the weight reduction program, but also between the changes in serum leptin level and the changes in ΣIRI after weight loss, was observed in all subjects as shown in Table 2 (r = 0.501, p < 0.001; HT: r = 0.602, p < 0.001; NT: r = 0.463, p < 0.001). In addition, a significant positive correlation was found between the changes in the log serum leptin and changes in log ΣIRI after adjusting for the changes in BMI (r = 0.463, p < 0.001), fat-mass (r = 0.441, p < 0.01), or total abdominal fat (r = 0.354, p < 0.01), respectively. The HOMA correlated significantly with log serum leptin not only at the beginning of the weight reduction program (r = 0.452, p < 0.001), but also after weight loss (r = 0.487, p < 0.001) in all subjects, any more than ΣIRI, respectively. As shown in Fig. 1, we found the significant positive single correlation between the change in log serum leptin and the changes in both MBP (r = 0.412, p < 0.05) and SBP (r = 0.389, p < 0.05) only in the HT group, whereas no correlation was observed in NT group. In addition, a significant partial correlation between the changes in MBP and the change in log serum leptin after adjusting for both changes in log ΣIRI and 24hU-NE was observed, but only in the HT group. In addition, to examine the relationship between changes in MBP and changes in log ΣIRI, 24h-NE, total abdominal fat and leptin after the 3-month weight reduction, a stepwise multiple linear regression analysis was performed. As shown in Table 3, four variables — total abdominal fat, log leptin, log ΣIRI, 24hU-NE — were selected as independent variables. The changes in log ΣIRI and 24hU-NE were determined to be significant predictive factors for a change in MBP after weight loss in all subjects. Regarding the standardized partial regression coefficients, the most important variable explaining the changes of MBP was the changes of the log leptin level in the HT group, but no correlation was observed between the changes in either log ΣIRI or 24hU-NE. After weight loss in four subject of the HT group, there was no decrease in blood pressure, no suppression of urinary NE levels, and either a decrease or no change in leptin levels (data not shown). In addition, we had to change the four variables from total abdominal fat, log leptin, log ΣIRI, 24hU-NE to total abdominal fat, log leptin, HOMA and 24hNE. Nevertheless, the changes in log serum leptin were determined to be a significant predictive factor for changes in MBP in the HT group after weight loss.

**Discussion**

The results of the present study indicate that the decrease in blood pressure after weight loss is probably related to the improvement of serum leptin level in obese hypertensive subjects. First, the serum leptin level in the HT group was significantly higher than that in the NT group before weight loss, after adjusting for either the BMI or abdominal total fat. Furthermore, we found a significant positive correlation between the changes in SBP, DBP and MBP and the change in log serum leptin in the HT group, but not in the NT group.
It has previously been reported that the decrease in blood pressure accompanying weight loss might be due to a reduction in insulin resistance and 24hU-NE (10, 22). In the present study, after weight loss, both the serum leptin and $\Sigma$IRI levels were found to have significantly decreased in all subjects. Furthermore, we also found a significant positive correlation between not only the log serum leptin levels and log $\Sigma$IRI at the beginning of the weight reduction program, but also between the changes in the log serum leptin level and the changes in log $\Sigma$IRI after weight loss. As for the role of leptin in the reduction of insulin resistance, insulin resistance in ob/ob mice has been reported to improve after the administration of leptin (23), although this effect has not yet been observed in humans. On the other hand, long-term hyperinsulinemia appears to increase both leptin production and the circulating leptin levels in humans (24). Such findings have suggested that leptin may thus play a role in the pathogenesis of insulin resistance in humans. However, there have been few reports dealing with the relationship of plasma leptin to hyperinsulinemia and blood pressure. Agata et al. (16) reported that the basal serum leptin level was significantly higher in an essential hypertensive group than in a normal group, and their findings were similar to those in the present study. However, they observed no significant relationship between the plasma leptin levels and insulin resistance in their normal weight subjects (BMI : NT, 23.9 ± 0.4 ; HT, 24.5 ± 0.4 kg/m$^2$), whereas we did observe a significant positive correlation between log leptin and both $\Sigma$IRI and HOMA. The differences between these results may be related to the difference in BMI levels between our study and that of Agata et al. (16). The plasma leptin were higher in hypertensive men but not in hypertensive women when compared with the normotensive control subjects (25, 26). However, such differences may be due to the relatively lower BMI levels in the former study (25) and the smaller sample size in the latter study (26). On the otherhand, Corica et al. very recently documented that leptin may play a role in the regulation of blood pressure in obese women (27).

Surprisingly, however, we found no significant correlation between either the changes in SBP or DBP and in log $\Sigma$IRI or HOMA and 24hU-NE after adjusting for changes in the log serum leptin levels during the 3 month weight reduction program (both expressed as a percentage of the levels at the start of the program) in the HT group. We found a significantly positive correlation between the changes in the log serum leptin concentration and those in SBP, DBP and MBP after weight reduction only in the HT group, but not in the NT group. This is the first prospective report on the association between changes in serum leptin and those in blood pressure in obese human subjects. Agata et al. reported that the plasma leptin level was closely correlated with the blood pressure in patients with essential hypertension in the baseline period, but they did not prospectively study the relationship between changes in leptin and those in blood pressure after weight loss in humans (16). However, it remains unclear why the leptin levels were elevated in the HT group even after adjusting for BMI. In a recent study on transgenic mice that overexpress leptin, Ogawa et al. reported the i.v. administration of leptin increase the SBP level via sympathetic activation without affecting the SBP in nontransgenic littermates (28). Eugene et al. reported that the chronic bilateral carotid artery or i.v. infusion of leptin during a 12-day period in rats increased the arterial pressure and heart rate, suggesting that leptin may play a role in obesity and hypertension (29). Recently, Aizawa-Abe et al. reported that the systolic blood pressure and urinary catecholamine excretion are elevated in transgenic skinny mice relative to those in nontransgenic littermates, while obese KKA$^\text{m}$ mice develop blood pressure elevation with an increased urinary catecholamine excretion relative to control KK mice. They hypothesized that leptin appears to be oversecreted from the adipose tissue of obese KKA$^\text{m}$ mice into the circulation, while in transgenic skinny mice leptin seems to be oversecreted from the liver into the circulation, thus causing a significant blood pressure elevation with increased catecholamine production, respectively. As a result, these findings indicate that a blood pressure elevation in transgenic skinny mice and KKA$^\text{m}$ mice, both of which are hyperleptinemic, thus suggesting that leptin may play a role in the pathogenesis of some forms of obesity-related hypertension (30). We thus consider that hyperinsulinemia may play an important role in blood pressure in moderately obese individuals, while serum leptin plays a role in obesity-related hypertensive individuals. In the present study, the serum leptin level decreased in both groups, but no correlation was observed between the changes in the log serum leptin concentration and those in SBP, DBP or MBP after weight reduction in the NT group. The significant blood pressure elevation with increased catecholamine production is therefore considered to be due to the increase in leptin; nevertheless, the urinary NE excretion decreased significantly in the HT group relative to the NT group. The effect of leptin itself on the blood pressure in humans is still unknown, and therefore future studies are needed.

Some of the potential limitations of the present study include: the subjects only consisted of women, the sample size in the HT group was small, and did not use the glucose clamp method. We also discussed insulin resistance using HOMA, and the insulin level using $\Sigma$IRI, but not the $M$-value.

In summary, obese hypertensives in the present study showed not only insulin resistance, but also to relatively hyper serum leptin levels. Furthermore, the changes in serum leptin levels correlated to the changes in blood pressure after weight loss only in the obese HT group. These findings thus suggested that leptin may play a role in the pathophysiology of obese hypertension.
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References


