Atrial Natriuretic Peptide and Leptin in Obesity-associated Hypertension in Middle-aged Egyptian Women

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ABSTRACT

Obesity is a world-wide health problem, whose incidence and prevalence are rising steadily; it may be combined with hypertension, diabetes, dyslipidemia, atherosclerosis, and/or chronic renal failure. Among these, hypertension has been observed in roughly 50% of obese individuals, which has led researchers to consider obesity as one of the most common causes of hypertension. The mechanisms linking obesity to hypertension have not been fully cleared. The present study aimed to clarify the interaction of leptin, soluble leptin receptors and the atrial natriuretic peptide in obese hypertensive patients. The study was performed on seventy five female subjects who were classified into three groups: group I (n=15) healthy lean controls, group II (n=30) obese normotensive patients, and group III (n=30) obese hypertensive patients. All participants were subjected to a thorough clinical assessment, and estimation of serum levels of glucose, creatinine, urea, cholesterol, leptin, soluble leptin receptors (sLR), and atrial natriuretic peptide (ANP). The serum levels of leptin and ANP were significantly higher in obese hypertensive and obese normotensive patients than in the controls, also, these levels were significantly higher in obese hypertensive patients when compared to obese normotensive patients. The serum sLR level in obese hypertensive and obese normotensive patients was significantly lower than in the controls, while its level in obese hypertensive patients was significantly lower than in obese normotensive patients. In the whole studied groups, the serum leptin and ANP levels were positively correlated, significantly, with BMI, diastolic blood pressure, and systolic blood pressure, but their levels were negatively correlated significantly with sLR. The serum sLR level was, significantly, negatively correlated with BMI, diastolic blood pressure, systolic blood pressure, leptin and ANP.

Keywords: Atrial natriuretic peptide, leptin, soluble leptin receptor, obesity, hypertension.

INTRODUCTION

With increasing the rate of obesity among adults and children, authorities view it as a serious public health problem. Between 1980 and 2000, obesity incidence among adults has more than doubled; and obesity among adolescents has tripled. In the USA, obesity is the second-leading
cause of preventable death after smoking (1). In Egypt, it has been reported that the prevalence of obesity in adults is very high, particularly among women, and that the prevalence of diabetes and hypertension parallels that of obesity (2).

Understanding of neuroendocrinial mechanisms regulating appetite metabolism and adiposity is revised since the discovery of leptin (3). Leptin is an adipocyte-derived hormone that acts on the hypothalamus to regulate appetite, energy expenditure, and sympathetic outflow. Leptin promotes weight loss by reducing appetite and by increasing energy expenditure through stimulation of sympathetic nerve activity (4). The excitement that followed leptin discovery was soon modulated by the realization that obesity is associated with hyperleptinemia, defining a state of “leptin resistance” (5).

Leptin plays its role by interaction with the specific leptin receptor. The leptin receptor exists in several isoforms. A soluble form of leptin receptor (sLR) represents the main leptin-binding activity in human blood. Circulating sLR is derived from ectodomain shedding of membrane-bound receptors and/or by alternative splicing of leptin receptor mRNA. It modulates steady-state leptin levels by modifying circulating free leptin to protect the hormone from degradation and clearance. Binding of leptin with soluble leptin receptor has been suggested to increase the bioavailability of leptin in plasma as well as to decrease binding of leptin to membrane specific receptors (6). When sLR is incubated with free leptin, the leptin-sLR complex is incapable of activating membrane-bound receptors, although it does not inhibit the action of free leptin (7).

The risk of hypertension is five-times higher in the obese subjects as compared to those of normal weight (8). The mechanisms linking obesity to the development of hypertension have not been well-identified (9).

Atrial natriuretic peptide (ANP) is a circulating neurotransmitter regulating salt and water homeostasis and vascular tone that control blood pressure. It is cardiac in origin and is secreted mainly in response to increased intra-atrial pressure (10). Abdel Hafez et al. (11) reported rise in plasma ANP in obesity, which became more elevated if the obesity was associated with hypertension.

The present work was planned to study the interaction of leptin and atrial natriuretic peptide in pathogenesis of hypertension in middle-aged obese females.

**SUBJECTS & METHODS**

Seventy-five female subjects were enrolled in this study. They were divided according to body mass index (BMI) and blood pressure (BP) into the following groups:

**Group I:** Healthy lean controls (n = 15), with age ranged between 22-47 years, BMI ≤ 25 kg/m² and BP ≤ 120/80 mmHg.

**Group II:** Obese normotensive patients (n = 30), with age ranged between 20-47 years, BMI ≥ 30 kg/m² and BP ≤ 120/80 mmHg.
Group III: Obese hypertensive patients (n = 30), with age ranged between 21-48 years, BMI ≥ 30 kg/m² and BP ≥ 140/90 mmHg. They presented with never-treated, uncomplicated essential (primary) hypertension.

All patients were recruited from the Outpatients' Clinic of Internal Medicine Section, Kasr Al-Aini Hospital, Cairo University. The control volunteers were females from the working staff. All participants gave their informed consent before participation in the study, and the Ethics Committee of Kasr Al-Aini Hospital approved the protocol of the study.

For all subjects, arterial blood pressure was measured in the right arm by mercury sphygmomanometer three times while subjects were in a complete physical and mental rest (i.e. sitting or supine for at least 5.0 minutes before measurement) in a quite room at ambient temperature. Average values were calculated for diastolic and systolic pressures, large cuff sizes were used in obese subjects. BP values ≤120/80 mmHg were considered normotensive, while values ≥ 140/90 mmHg were considered hypertensive (12).

Detailed history taking, physical examination and laboratory investigations were done to exclude acute illness, cardiac, hepatic, renal, gastrointestinal, endocrinial, or malignant disease that might affect the parameters under investigation. None of the patients was receiving drugs known to affect the investigated parameters.

To minimize the effect of sex and age on the results of this work, all subjects were females aged 20-47 years old and not receiving contraceptive or hormonal therapy. Blood sampling was performed early in the follicular phase of the menstrual period (within the first week of the cycle).

Analytic procedures:

Following an overnight fast (12 hours), 10 ml blood was collected from the antecubital vein, and serum was kept frozen, in aliquots, at -20°C until used. The following laboratory investigations were performed for each subject: fasting blood glucose (13), serum creatinine (14), serum urea (15), total cholesterol (16), serum leptin by RIA technique (LINCO Research, Missouri, USA) (17), serum soluble leptin receptors (sLR) by ELISA method (R&D Systems, Minneapolis, USA) (18), and serum atrial natriuretic peptide (ANP) by RIA technique (Phoenix Pharmaceuticals Inc., Burlingame, California, USA) (19).

Statistical Methods:

The results were analyzed using Statistical Package for Social Science (SPSS) program version 10 (Chicago-IL, USA) (20). Data were presented as mean ± S.D. Student t-test was used for analysis of two quantitative data. Differences among the three groups were compared by one-way ANOVA followed by post-hoc test. Simple linear correlation (Pearson’s correlation for quantitative data and Spearman correlation for qualitative data) was done to detect the relation between leptin, sLR and ANP with all other demographic and laboratory data.
RESULTS

The clinical characteristics and anthropometric measurements (mean ± SD) in healthy control (Group I), obese normotensive (Group II) and obese hypertensive subjects (Group III) are shown in Table I.

Table I: Clinical characteristics and anthropometric measurements of the studied groups (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.40 ± 13.6</td>
<td>35.43 ± 8.88</td>
<td>38.00 ± 7.49</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.25 ± 1.10</td>
<td>43.04 ± 9.54a</td>
<td>43.46 ± 5.73a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74.00 ± 5.07</td>
<td>74.66 ± 5.07</td>
<td>92.66 ± 3.82ab</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>116.00 ± 5.07</td>
<td>116.33 ± 6.14</td>
<td>146.00 ± 7.78ab</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BP: Blood pressure, BMI: Body mass index, p<0.05 was considered significant, (a) Significant difference versus control subjects (b) Significant difference versus obese normotensive patients

The serum levels of creatinine, leptin and ANP were significantly higher in groups II and III versus group I. Also, these levels were significantly higher in group III when compared to group II (Table II).

Table II: Laboratory data of the studied groups (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. Glucose (mmol/l)</td>
<td>4.19 ± 0.19</td>
<td>4.98 ± 0.46a</td>
<td>5.07 ± 0.73a</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>4.53 ± 0.73</td>
<td>6.96 ± 0.96a</td>
<td>7.49 ± 0.85a</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>96.8 ± 28.1</td>
<td>118.8 ± 20.2a</td>
<td>141.7 ± 28.1ab</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.11 ± 0.13</td>
<td>5.08 ± 0.25a</td>
<td>4.85 ± 0.41a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>6.22 ± 1.64</td>
<td>31.03 ± 8.13a</td>
<td>49.00 ± 12.2ab</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sLR (ng/ml)</td>
<td>37.26 ± 6.69</td>
<td>26.93 ± 6.94a</td>
<td>20.8 ± 4.17ab</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>53.7 ± 11.98</td>
<td>136.3 ± 35.69a</td>
<td>250.6 ± 94.75ab</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

p<0.05 was considered significant, (a) Significant difference versus control subjects (b) Significant difference versus obese normotensive patients

Serum leptin levels in the studied groups were positively correlated significantly (P<0.001) with BMI (Figure 1) (r=0.680), diastolic blood pressure (r=0.612), systolic blood pressure (r=0.614), cholesterol (r=0.380), urea (r=0.608), creatinine (r=0.390) and ANP (r=0.714) (Figure 2). While it was negatively correlated significantly with sLR (r= -0.817) (Figure 3).
Figure 1: Scatterplot showing correlation between serum Leptin and BMI in the studied groups (r=0.680, p<0.001)

Figure 2: Scatterplot showing correlation between serum leptin and ANP in the studied groups (r=0.714, p<0.001)

Figure 3: Scatterplot showing correlation between serum Leptin and sLR in the studied groups (r=-0.817, p<0.001)

The serum sLR was negatively correlated significantly (P<0.001) with BMI (r= -0.540) (Figure 4), diastolic blood pressure (r= -0.554), systolic blood pressure (r= -0.557), cholesterol (r= -0.347), urea (r= -0.540), creatinine (r= -0.345), leptin (r= -0.817) (Figure 3) and ANP (r= -0.550).

Figure 4: Scatterplot showing correlation between serum sLR and BMI in all studied groups (r=-540, p<0.001)
Serum ANP levels of the investigated groups were, significantly (P<0.001) positively correlated with BMI (r=0.525) (Figure 5), diastolic blood pressure (r=0.561), systolic blood pressure (r=0.570), cholesterol (r=0.256), urea (r=0.536), creatinine (r=0.431) and leptin (r=0.714) (Figure 1). While it was significantly negatively correlated with sLR (r= -0.550, P<0.001).

**DISCUSSION**

Leptin, as adipocytokine is derived from adipose tissue, it is significantly elevated in obese groups versus lean control group, with significant positive correlation between serum leptin concentration and BMI in all studied groups.

Mackintosh and Hirsch (21) reported that females with increased fat mass are associated with higher baseline leptin levels and endogenous production rates. Several other studies demonstrated that increased adiposity is correlated with increased serum leptin levels secondary to increased production rate and decreased elimination (22,23). In addition, Hafeezullah (24) confirmed increased production rate through reporting higher concentration of leptin mRNA in fat from obese compared to thin subjects. The latter finding suggests that leptin gene is expressed more in adipose tissue of obese than from lean subjects. The underlined mechanism affecting the rate of gene expression is not clear.

In the present study the serum levels of leptin were significantly higher in group III than in groups II and I. Also, a significant positive correlation was detected between serum leptin and both systolic and diastolic blood pressures in all studied groups. This suggests that blood pressure might be affected by circulating leptin, or a common effector on leptin level and blood pressure is playing a role. Al-Hazimi and Syiam (25) suggested that serum leptin and angiotensin II levels were strong predictors of elevated blood pressure in obese women.

El-Gharbawy and co-workers (26) found that leptin levels were higher in hypertensive than in normotensive African Americans, but once these individuals were adjusted for obesity, no significant relationship was further observed between leptin and blood pressure. Other investigators, Shankar and Xiao (27), reported higher plasma leptin that was positively correlated with hypertension, even after adjusting for other factors. Reduction in serum...
leptin levels with administration of angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers suggest a potential interaction between leptin and the renin-angiotensin-aldosterone system for hemodynamic regulation in obesity\(^{(28)}\). Hyperleptinemia enhances vascular smooth muscle cell proliferation\(^{(29)}\). Leptin increases the accumulation of reactive oxygen species (ROS) in endothelial cells and thereby might play a major role in initiation of an inflammation process and genesis of vasoconstriction and atherosclerosis\(^{(30)}\). Leptin may function as a pressure and volume-regulating factor in health and obesity-linked hyperleptinemia, resulting in development of hypertension\(^{(31)}\).

Leptin activates the sympathetic nervous system by both local peripheral actions as well as through centrally mediated effects on the hypothalamus\(^{(32,33)}\). The "selective leptin resistance" theory is feasible to explain why hyperleptinemia contributes to increased sympathetic activity and arterial pressure in obesity, in spite of resistance to the metabolic (satiety and weight-reducing) actions of leptin\(^{(34)}\).

Besides sympathetic activation, leptin decreases renal vascular nitric oxide (NO) production resulting in systemic vasoconstriction along with sodium retention\(^{(35)}\). Serum creatinine and urea in our obese groups are higher than the lean group suggesting decrease renal efficiency in obesity. Lack of NO and abnormal renal Na\(^+\) handling may contribute to leptin-induced hypertension\(^{(36,37)}\). Hyperinsulinemia characterizing obesity may be a contributing factor in development of sodium retention\(^{(38)}\). Conclusively, the interaction among the vasoconstricting, vasodilatory, and natriuretic effects of leptin to achieve volume and pressure homeostasis in normal conditions may be disrupted during chronic hyperleptinemia, and this effect could lead to hypertension and possible cardiac and renal dysfunction\(^{(39,40)}\).

In the present study the sLR serum levels were significantly lower in obese hypertensive and obese normotensive patients compared to the control group. Similar results were obtained by\(^{(41,42,43)}\). The relationship of sLR with the degree of adiposity suggests that high sLR levels may enhance leptin action in lean subjects more than in obese subjects\(^{(34)}\), and in lean subjects leptin circulates mainly in the bound form, whereas in obese subjects the majority of leptin circulates in the free form\(^{(1)}\).

In the present study, there was a significant negative correlation between serum sLR and both BMI and leptin in the whole studied population. Circulating sLR expresses how leptin receptors are bioactive. In obesity, characterized by hyperleptinemia and leptin resistance, there may be down-regulation of leptin receptors, resulting in reduced circulating sLR\(^{(45)}\). Huang et al.\(^{(42)}\) and Gajewska et al.\(^{(6)}\) had reported that the sLR showed a significant inverse correlation with leptin and percentage of body fat.

Because the natriuretic peptide system plays a key role in the regulation of renal handling of sodium and water, it has been speculated that obese individuals may have an
impaired natriuretic peptide response. In a study in our laboratory, raised serum ANP was observed in obese subjects with and without hypertension (41).

In the present study, ANP levels of obese hypertensive and obese normotensive subjects had significantly higher serum ANP level than that of the lean control subjects. Also, the mean serum ANP level of obese hypertensive subjects was significantly higher than ANP level of obese normotensive. There was a significant positive correlation between serum ANP level and systolic BP, diastolic BP and BMI in the whole studied population.

Numerous studies reported high concentrations of ANP in patients with arterial hypertension regardless of body weight (46,47,48).

Rise of serum ANP in obesity may be a pathophysiologic mechanism to protect against the hemodynamic changes that are associated with expanded blood volume in obesity and hypertension (41). ANP is known to be vasodilator and an inhibitor to rennin-angiotensin-aldosterone axis, besides its diuretic and natriuretic effects (49).

In the study of Wang and co-workers (50), plasma natriuretic peptide levels were, variably, higher in hypertensive compared with normotensive individuals. However, in contradiction to our results they reported that overweight and obese individuals with hypertension still had lower plasma ANP level compared with normotensive individuals with normal BMI. Natriuretic peptides, in obese patients with hypertension may protect against further elevation of blood pressure rather than contribute to the pathogenesis of hypertension by guarding against fluid volume expansion.

In this study, a significant positive correlation was detected between serum ANP level and leptin, while a significant negative correlation was found between serum ANP level and sLR in the whole studied groups. Such correlation suggests that ANP may influence leptin receptor activity. Both leptin and ANP enhance sympathetic over-activity (51).

Atrial natriuretic peptide has been shown to promote adipose tissue lipolysis through cGMP-mediated hormone-sensitive lipase activation (52,53). Nielsen et al. (54) suggested that the increased release of free fatty acids (FFA) into the portal vein from lipolysis in visceral fat depots of obese subjects could explain the strong association between visceral obesity and increased sympathetic nerve outflow and blood pressure. Obesity-associated hyperinsulinemia may be a crucial pathophysiologic feature of promoted lipolysis in visceral adipose tissue (55). Fatty acid binding protein-2 may undergo mutation in metabolic syndrome, this impaired fatty acid utilization may ensue resulting in enhanced sympathetic activity (56).

In addition, Mascarenos et al. (57), investigated the molecular mechanism(s) that play a role in leptin signaling during the development of left ventricular hypertrophy due to pressure overload (i.e. hypertension), and they observed that leptin plays a role in modulating the transcriptional activity of nuclear factor activated T
cell 4 which is the promoter of the ANP gene, followed by an increase in the expression of the ANP gene. Increased ANP in patients with idiopathic hypertension might indicate left ventricular hypertrophy, in which the increased left ventricular mass may significantly enhance the ventricular contribution of circulating ANP (58).

Thus, it seems that an interaction between leptin and ANP in development of hypertension in obese subjects may exist. The interaction may aim at reduction of the circulating plasma volume besides adapting for energy homeostasis.

Serum glucose in obese groups is significantly higher than the lean control group. Insulin has a sustained antinatriuretic action that is triggered by increased glucose, and it is powerful enough to completely block the natriuresis caused by hyperglycemia (38). This action of insulin may be an etiological factor of sodium retention in obesity with subsequent rise of blood pressure.

To summarize, many hormonal factors are interplaying to cause rise of blood pressure in obesity; leptin through enhancing sympathetic activity and affection of the renal functions. Leptin, also, promotes release of ANP from ventricles to modulate plasma volume, vascular tone, renal water and sodium homeostasis. Both promote lipolysis from visceral adipose tissue. The latter effect is enhanced by hyperinsulinemia characterizing obesity. ANP, along with leptin and FFA activates sympathetic functions.

REFERENCES


البحث الدوالي المدر للصوديوم والليبتين في حالات ارتفاع ضغط الدم

المصاحبة للبدانة في السيدات المصريات متوسطات العمر

محمد علي عبد الحافظ، خالد حمدي، حمد الصقلي

سفر على مروي، مروي فتحي

قسم الكيمياء الحيوية الطبية والبيولوجيا الجزيئية 1

قسم الأمراض الباطنة 2

كلية الطب، جامعة القاهرة

البدانة مشكلة صحية في جميع أنحاء العالم حيث أن جذورها وانتشارها تزيد باطراف، وهي من الممكن أن تتج مع ارتفاع ضغط الدم، مرضى البدانة السكري، اختلالات دهون الدم، تصل إلى التشريعا أو القليل الكليوي المزمن. من بين هؤلاء، وفور أن ارتفاع ضغط الدم يوجد في حوالي 25% من الأفراد البيئيين، مما قد يمثل نوعية أولية للبدانة أو نوعية مشروعة للإصابة بالبدانة.

الجزء الأول: أنواع الضغط في ارتفاع ضغط الدم في الآلالات التي ترتبط البدانة بالضغط الدم، فلم يتم إيضاحها بالكامل. تهدف الدراسة الحالية إلى توضيح التفاعلات بين البدانة، مستويات البدانة، مستويات البدانة، مستويات البدانة، مستويات البدانة، مستويات البدانة، مستويات البدانة.

البدانة: هذه الدراسة تم تنفيذها على 15 سنة، حيث تم تضمينها إلى ثلاث مجموعات: المجموعة الأولى (عدد 30) مجموعات متوسطة ضغط متوسط معيّن، المجموعة الثانية (عدد 30) مجموعات متوسطة ضغط متوسط معيّن، المجموعة الثالثة (عدد 30) مجموعات متوسطة ضغط متوسط معيّن

بينما نستكمل هذه المجموعات في الدراسة، فإن تقييم البدانة بشكل عام، وتفضيل مستوى مرن مطلق للبدانة، مرن مطلق للبدانة، مرن مطلق للبدانة، مرن مطلق للبدانة، مرن مطلق للبدانة.

وقد أن مستويات مرن مطلق للبدانة والبدانة الدوالي للصوديوم قد أوضحت زيادة ذات دلالة

البدانة في مجموعتي البدانة وضغط الدم الطبيعي والبدانة وضغط الدم المرتفع مقارنة بالجمعية السابقة، كما كانت هناك زيادة ذات دلالة إحصائية في مجموعتي البدانة وضغط الدم المرتفع مقارنة بالجمعية précédentية. أما مستويات مستقبلات البدانة، ففي مجموعتي البدانة وضغط الدم الطبيعي والبدانة، فقد أوضحت ارتفاع ضغط الدم الفضائي للبدانة في مجموعتي البدانة وضغط الدم الطبيعي والبدانة، وتبعاً لذلك، وضعت البدانة المرتفعات:

في كل المجموعات التي تمّت دراستها، وجد أن مستويات البدانة والبدانة الدوالي للصوديوم في مجموعتي البدانة، وضغط الدم المرتفعات. أما مستويات البدانة، ففي مجموعتي البدانة، والتي توضع فضائيًا للبدانة، وضغط الدم المرتفعات. أما مستويات البدانة، ففي مجموعتي البدانة، والتي توضع فضائيًا للبدانة، وضغط الدم المرتفعات. أما مستويات البدانة، ففي مجموعتي البدانة، والتي توضع فضائيًا للبدانة، وضغط الدم المرتفعات.

أما عن مستويات مستقبلات البدانة الفضائية للبدانة في مجموعتي البدانة، وضغط الدم المرتفعات، وضغط الدم المرتفعات، وضغط الدم المرتفعات، وضغط الدم المرتفعات، وضغط الدم المرتفعات، وضغط الدم المرتفعات.

إن البدانة والبدانة الدوالي للصوديوم قد ينكو على عملية نضح الكلي وبالتالي يؤدي إلى اقتصاد الماء والصوديوم. كما أنهما هذامون قد ينكو على الجهاز السينتاث ووجه البدانة، في ارتفاع ضغط الدم في حالات البدانة.