Elevated circulating leptin levels in arterial hypertension: relationship to arteriovenous overflow and extraction of leptin

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ABSTRACT

Leptin, a peptide hormone produced mainly in fat cells, appears to be important for the regulation of metabolism, insulin secretion/sensitivity and body weight. Recently, elevated plasma leptin levels have been reported in patients with arterial hypertension. Because a change in circulating leptin concentrations in such patients could be caused by altered rates of production or disposal, or both, the aim of the present study was to identify regions of leptin overflow into the bloodstream and of leptin extraction. Patients with arterial hypertension (n = 12) and normotensive controls (n = 20) were studied during catheterization with elective blood sampling from different vascular beds (artery, and renal, hepatic, iliac and cubital veins). Plasma leptin was determined by a radioimmunoassay. Patients with hypertension had significantly elevated levels of circulating leptin (12.8 ng/l, compared with 4.1 ng/l in the controls; P < 0.001), and this was also the case when adjusted for body mass index (BMI) [0.435 and 0.167 ng/l per unit BMI (kg/m^2) respectively; P < 0.001]. Circulating leptin was directly related to arterial blood pressure (r = 0.38–0.62, P = 0.05–0.005) and immunoreactive insulin (r = 0.51, P = 0.02), but not to plasma renin activity. A significant renal extraction ratio for leptin was seen in the hypertensive patients, but this was not significantly lower than that in the controls (0.09 compared with 0.16; P = 0.1). The hypertensive patients had a significantly higher hepatic venous/arterial leptin ratio than the controls (1.02 compared with 0.93; P = 0.02), and this ratio was correlated directly with the BMI (r = 0.38, P = 0.05) and immunoreactive insulin (r = 0.43, P = 0.05). In both hypertensive patients and controls there was a significant spillover of leptin into the iliac vein, but not into the cubital vein. In conclusion, the high concentration of circulating leptin in patients with arterial hypertension is probably caused by increased release of leptin from abdominal (especially mesenteric and omental) and gluteal adipose tissue stores, and renal extraction is slightly reduced. Leptin kinetics in arterial hypertension require further investigation.

INTRODUCTION

Leptin, a peptide hormone produced in fat cells, was discovered in 1994 by Friedman and co-workers [1]. It decreases appetite and food intake, increases the expenditure of energy, and appears to be important for the regulation of metabolism, insulin secretion/sensitivity and body weight [1–6]. Circulating leptin is related to the

Key words: abdominal adipose tissue, arterial blood pressure, body mass index, extraction ratios, hypertension, insulin, leptin, overflow.

Abbreviation: BMI, body mass index.

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adipose tissue mass, and in most subjects the levels correlate directly with the body mass index (BMI) [2,6]. The circulating leptin concentration is higher in normal women than in men [5,6]. In addition to the adipose tissue mass, leptin production is affected by glucose and steroid metabolism [1,2,4,5], and non-adipose sources of leptin have now been documented [7–9].

High circulating levels of leptin have been reported in patients with diabetes, renal insufficiency and cirrhosis [10–14]. Elevated plasma concentrations of leptin have been also described in patients with arterial hypertension [15–18], and there are indications that high plasma leptin may play a pathophysiological role in arterial hypertension [16,19,20] and stroke [21].

As elevated circulating levels of leptin in arterial hypertension could be caused by an increased production rate, a decreased disposal rate, or both, the present study was undertaken to identify vascular regions of leptin overflow into the bloodstream and regions of leptin extraction. We studied patients with arterial hypertension and control subjects with normal blood pressure during venous catheterization with elective blood sampling in different vascular beds. Moreover, the relationships of circulating leptin to body size, homoeostatic mechanisms and systemic haemodynamics were also examined.

METHODS

Study population

The study population comprised 12 patients (three women and nine men) with arterial hypertension. All patients were suspected of secondary hypertension and were therefore referred for the collection of elective renin samples from artery and renal veins during a haemodynamic investigation. Eleven patients had essential hypertension, and one patient had renovascular hypertension with excessive generation of renin from the left kidney. Two patients with essential hypertension had a contracted kidney on one side with low function, as assessed by isotope renography. All the patients had normal serum creatinine. The age range was 39–72 years (mean 56 years). All the patients had a history of increased arterial blood pressure (suspicion of intestinal ischaemia, although unconfirmed, irritable bowel syndrome, postcholecystectomy pain) who underwent diagnostic catheterization plus eight normal subjects served as controls [10]. The age range was 45–79 years (mean 56 years). Of the controls, five were women and 15 were men.

All the subjects consented to participate in the study, which was approved by the Ethics Committee for Medical Research in Copenhagen (KF 01-243/98) and performed in accordance with the guidelines established in the Declaration of Helsinki II. No complications or side effects were encountered during the study.

Catheterization

Catheterization was performed in the morning after an overnight fast and after the subject had rested for at least 1 h in the supine position, as described elsewhere [22]. Briefly, a Cournand catheter (7F) or Swan-Ganz catheter (7F) was guided under local anaesthesia to the renal and hepatic veins through the femoral route under fluoroscopy. A small indwelling polyethylene catheter (5F) was introduced into the femoral artery by the Seldinger technique. Simultaneously, paired blood samples for leptin determination were taken from the following locations: artery/renal vein, artery/hepatic vein, artery/iliac vein and artery/cubital vein, as described previously [10,22,23].

The renal ($E_r$) and hepatosplanchnic ($E_h$) extraction ratios for leptin were determined as $E_r = (C_a - C_{rv})/C_a$ and $E_h = (C_a - C_{hv})/C_a$, where $C_a$, $C_{rv}$ and $C_{hv}$ are the leptin concentrations in arterial, renal and hepatic venous plasma.

Pressures were measured with a capacitance transducer (Simonsen & Weel, Copenhagen, Denmark), as described previously [22,24]. Frequency characteristics and the reliability of dynamic intravascular pressure measurement, including determination of systolic and diastolic pressures, have been evaluated with this equipment [24]. The mean arterial pressure was determined by electronic integration of the pressure signal. Right atrial pressure was determined as the mean pressure over 15 s. Zero reference was the mid-axillary level.

Cardiac output and plasma volume were measured by the indicator dilution technique after a bolus injection of 150 KBq of $^{131}$I-labelled human serum albumin into the right atrium, as described previously [25]. Systemic vascular resistance was determined as: 

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\frac{\text{[\text{mean arterial pressure} - \text{right atrial pressure}]}}{\text{cardiac output}}
\]

\[24\]
Biochemical analysis
Routine biochemical tests were performed using an autoanalyzer (SMAC; Technicon Instruments, Tarrytown, NY, U.S.A.) and a Beckman Glucose Analyzer 1 (Beckman Instruments Inc., Fullerton, CA, U.S.A.). Plasma renin activity (Sanofi Pasteur, Marnes LaCoquette, France) and plasma aldosterone concentration (MedLab, Copenhagen, Denmark) were determined with commercially available radioimmunoassays.

Immunoreactive insulin in plasma was measured according to the principles described by Albano et al. [26] against standards of human insulin. The tracer was human insulin monoiodinated in position A14 (a gift from Novo-Nordisk A/S, Bagsværd, Denmark), and the antibody was from guinea pig (code no. 2004) [10]. The interassay coefficient of variation was below 5%, and the detection limit was below 5 pmol/l.

Blood samples for leptin analysis were collected in prechilled tubes (6 units of heparin/500 kallikrein-inhibitory units of aprotinin per ml), centrifuged at 4 °C and stored at −25 °C until analysed. The analysis of leptin was performed by a radioimmunoassay using a commercially available kit for human leptin (catalogue no. 25,625; Linco Research, Inc., St. Louis, MO, U.S.A.), as described previously [10,27]. The assay was linear in the range 0.5–100 ng/ml. For the batches utilized in the present study, all high and low control samples provided by the manufacturer conformed with expected values, and the standard curves showed ED_{20}, ED_{50} and ED_{80} values (doses giving 20%, 50% and 80% respectively of the maximum response), as indicated by the manufacturer. Intra- and inter-assay coefficients of variation ranged from 3.4 to 6.2% and from 3.6 to 8.3% respectively, at a concentration range between 1.9 and 25.6 ng/ml. The detection limit in our hands was 0.5 ng/ml.

Statistical evaluation
Data are expressed as means ± S.E.M. Statistical analysis was performed by unpaired/paired Student’s t test or the Mann–Whitney/Wilcoxon rank tests. Correlations between independent variables were performed with the Pearson regression test (method of least squares) or by Spearman’s rank correlation test. P ≤ 0.05 was considered to be significant.

RESULTS
Clinical, biochemical and haemodynamic data are summarized in Table 1. Arterial blood pressure and systemic vascular resistance were substantially increased, but no significant differences were recorded in heart rate, cardiac output or plasma volume in the hypertensive patients compared with the controls. Circulating immunoreactive insulin was higher in the patients with hypertension than in the controls (242 and 130 pmol/l respectively; P < 0.001).

Circulating leptin levels are summarized in Table 2 and Figure 1. The hypertensive patients had substantially elevated leptin levels (12.8 ng/l, compared with 4.1 ng/l in the controls; P < 0.001), and this was also the case...
Table 2  Circulating leptin levels in 12 patients with arterial hypertension and 12 normotensive controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Arterial leptin (C_a) (ng/l)</th>
<th>Peripheral venous leptin (ng/l)</th>
<th>Arterial leptin relative to BMI [ng/l per unit BMI (kg/m²)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive patients</td>
<td>12.8 ± 2.5***</td>
<td>12.9 ± 2.4***</td>
<td>0.435 ± 0.087***</td>
</tr>
<tr>
<td>Controls</td>
<td>4.1 ± 0.5</td>
<td>4.0 ± 0.5</td>
<td>0.167 ± 0.016</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E.M. Significance of differences: *** P < 0.001 compared with controls.

Figure 1  Plasma leptin concentrations in control subjects and in patients with arterial hypertension

Circulating leptin concentrations in controls (n = 20) and in patients with arterial hypertension (n = 12) are shown. Closed symbols indicate subjects with BMI in the range 22–29 kg/m²; open symbols denote subjects with a BMI outside this range; ▲, patients with essential hypertension with unilateral contracted kidney; ◯, patient with renovascular hypertension with unilateral renin generation.

Figure 2  Overflow and extraction of leptin

Venous/arterial ratios (C_v/C_a) of leptin in different vascular beds are shown. A ratio below 1.00 indicates an area of net leptin disposal, and a ratio above 1.00 indicates an area of net leptin spillover into the circulation. ■, Hypertensive patients; □, controls; numbers indicate the number of subjects providing simultaneous arterial and venous samples. Significance of differences compared with a ratio of 1.00: * P < 0.05; ** P < 0.001.

Table 3  Arteriovenous extraction ratios in the renal and hepatosplanchnic vascular beds in 12 patients with arterial hypertension and 12 normotensive controls

Extraction ratios were calculated as follows: E_r = (C_a - C_r)/C_a and E_h = (C_a - C_h)/C_a (see the text for details). Data are expressed as means ± S.E.M. Significance of difference: ** P < 0.02 compared with controls.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Renal extraction (E_r)</th>
<th>Hepatosplanchnic extraction (E_h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive patients</td>
<td>0.09 ± 0.034</td>
<td>0.021 ± 0.025*</td>
</tr>
<tr>
<td>Controls</td>
<td>0.16 ± 0.021</td>
<td>0.07 ± 0.027</td>
</tr>
</tbody>
</table>

when expressed relative to the BMI [0.435 compared with 0.167 ng/l per unit BMI (kg/m²), P < 0.001]. Similarly, in the BMI range 22–29 kg/m², the mean leptin level in the hypertensive patients (n = 9) was 11.1 ng/l, compared with 4.9 ng/l in the controls (n = 11) (P < 0.05) (see Figure 1). Circulating leptin was directly related to body weight and BMI in the hypertensive patients (r = 0.87, P < 0.001 and r = 0.89, P < 0.001 respectively) and in the controls (r = 0.65, P < 0.05 and r = 0.84, P < 0.001 respectively).

Circulating leptin was directly related to systolic pressure (r = 0.62, P < 0.005), diastolic pressure (r = 0.38, P = 0.05) and mean arterial pressure (r = 0.56, P < 0.01) when controls and hypertensive patients were analysed together, but significance was not reached in the two separate groups. The level of circulating leptin did not correlate with heart rate (excluding the two patients on β-blockers), plasma volume, cardiac output, plasma aldosterone concentration or plasma renin activity, but there was a direct correlation with immunoreactive insulin (r = 0.51, P < 0.02).

The results of the simultaneous arteriovenous sampling are summarized in Figure 2. Significant renal extraction of circulating leptin was found both in the hypertensive patients and in the controls (P < 0.02 and P < 0.001 respectively). Renal extraction was slightly, but not
significantly, lower in the hypertensive patients than in the controls ($E_v = 0.09$ compared with 0.16; $P = 0.1$) (Table 3). Significant extraction of circulating leptin occurred in the hepatosplanchnic bed in the controls ($E_h = 0.07$), but not in the hypertensive patients ($E_h = -0.02$), who had a significantly higher hepatic venous/arterial leptin ratio than had the controls (1.02 and 0.93 respectively; $P < 0.02$) (Figure 2). In addition, there was a direct relationship between the hepatic venous/arterial leptin ratio on the one hand and BMI and immunoreactive insulin on the other ($r = 0.38$, $P = 0.05$ and $r = 0.43$, $P < 0.05$ respectively) (Figure 3).

A significant spillover of leptin into the iliac vein could be demonstrated in both the hypertensive patients and the controls (Figure 2). No significant leptin spillover was found in the cubital vein, either in the hypertensive patients or in the controls.

**DISCUSSION**

The present results show the following: (1) confirmation of elevated circulating leptin levels in hypertensive patients; (2) significant renal extraction of leptin is identified in hypertensive patients, but it is not significantly lower than that in controls; (3) the hepatic venous/arterial leptin ratio is increased in hypertensive patients; (4) significant spillover of leptin into the iliac vein occurs in both hypertensive patients and controls; and (5) elevated circulating leptin levels and hepatic venous/arterial leptin ratios are related to indicators of adipose tissue mass, blood pressure and immunoreactive insulin, but not to plasma renin, plasma aldosterone, plasma volume, heart rate or cardiac output.

The present study confirms that patients with arterial hypertension have increased circulating leptin levels [15,17,18,28]. Patients and controls were matched for age and height, but the hypertensive patients had a higher body weight and BMI, which, owing to their increased mass of adipose tissue, could in itself contribute to higher plasma leptin levels [1,2,6]. Circulating leptin was directly correlated with BMI in both controls and hypertensive patients, but the latter group had a higher level. In line with this, when comparing patients and controls in the BMI range 22–29 kg/m$^2$, the hypertensive patients still had substantially elevated levels of circulating leptin. This suggests that hypertensive patients have elevated circulating leptin that is both dependent on and independent of their fat mass. However, this conclusion is based on a univariate analysis, as the size of our study population did not allow a multivariate analysis. Two patients with essential hypertension and a contracted kidney on one side had very high values of circulating leptin (Figure 1). This may, in part, be caused by reduced renal disposal of leptin (see below). The possibility that the anti-hypertensive treatment may have contributed to the elevated levels of circulating leptin cannot be ruled out.

However, Agata et al. [15] and Narkiewicz et al. [18] reported high plasma leptin levels in untreated patients with essential hypertension. Thus, from the present and other studies, it can be concluded that patients with arterial hypertension have increased circulating leptin levels, irrespective of anti-hypertensive treatment. Sheu et al. [29] have recently reported that high plasma leptin concentrations are present in hypertensive men, but not in hypertensive women. This is supported by our results, as our study population was mainly men.

In our study, the renal extraction of circulating leptin was significant in both the hypertensive patients and the controls, as shown by the lower renal venous concentration compared with the arterial concentration when determined simultaneously. Moreover, the renal extraction in our control subjects ($E_r = 0.16$) was very similar to that found by Esler et al. [7] ($E_r = 0.17$). The hypertensive patients had a somewhat lower renal extraction ratio ($E_r = 0.09$), but the difference did not reach statistical significance; given the relatively small number of patients, this may represent a type II error. Where there was a difference in function between the left and the right kidney, as evaluated by isotope renography, the extraction ratio refers to the kidney with normal function. In the two patients with a contracted kidney on one side, the decrease in the renal disposal of leptin may, at least in part, have contributed to the marked elevation of circulating leptin [13,30,31]. The possibility that decreased renal disposal may, to some extent, contribute to the elevated circulating leptin in the other patients with
arterial hypertension is a likely one, as it is well known that renal perfusion in such patients is often somewhat reduced as compared with that of normotensive subjects [32]. This is consistent with experimental evidence for the renal tubular degradation of leptin, and findings of elevated circulating leptin in patients with organic renal disease and hepatorenal dysfunction [2,10–13]. Unfortunately we did not measure renal perfusion, which would allow determination of plasma clearance [33].

Normal animals and humans have a net disposal of circulating leptin by the integral hepatosplanchnic system [2,10]. This probably involves release from intestinal and omental adipose tissue, with subsequent extraction of leptin in the liver, analogous to numerous other peptides [10,33]. Accordingly, our control subjects had a significant net extraction of circulating leptin by the hepatosplanchnic system. By contrast, more than half of the hypertensive patients had a higher hepatic venous than arterial concentration of leptin, which indicates that no net disposal, but rather a net spillover, of circulating leptin occurs through the hepatic veins. This is most likely due to increased release of leptin from intra-abdominal mesenteric and omental adipose tissue, in combination with normal hepatic extraction of leptin, as our patients with arterial hypertension had no history or signs of hepatic dysfunction. In keeping with this view, a direct relationship was found between the hepatic venous/arterial ratio of leptin and the BMI, as it is well established that BMI generally reflects intra-abdominal adipose tissue mass [34].

We identified a significant spillover of leptin into the iliac veins of both hypertensive patients and control subjects, and the iliac venous/arterial ratio was similar in the two groups. This suggests that a substantial spillover of leptin, coming from abdominal retroperitoneal pelvic, gluteal and femoral adipose tissue, contributes to the circulating leptin. In the present study, both the hypertensive patients and the controls were fasting. We have recently demonstrated a small short-term variation in circulating leptin that is not significantly affected by the ingestion of food [10]. The present differences in vascular concentrations are therefore not likely to be caused by differences in the pattern of food intake between hypertensive patients and controls [35]. Unlike Suter et al. [17], we did not find any significant relationship between plasma renin activity and circulating leptin. The reason may be that our patients were treated with drugs, which may affect the plasma renin activity.

The circulating leptin concentration is not a static reflection of body stores of adipose tissue, and the expression and secretion of leptin are affected by actual metabolic and physiological signals, such as blood glucose, fatty acids and steroid hormones [36–41]; in addition, leptin may originate from non-adipose sources [7–9]. A relationship between circulating leptin and immunoreactive insulin has been demonstrated in normal subjects, obese subjects and patients with hypertension [2,4,38,42]. The present study is no exception, as we also found a direct relationship between the circulating levels of these two peptides. As a part of the metabolic syndrome (comprising obesity, Type II diabetes, hyperlipidaemia and hypertension), the implication of insulin resistance/hyperinsulinaemia in the pathogenesis of essential hypertension has been suggested [43]. Like insulin, leptin has actions on the sympathetic nervous system and renal sodium metabolism in rats that may be relevant in the pathogenesis of hypertension [19]. In rats, acute intracerebral infusion and chronic intravenous infusion of leptin raised the blood pressure and heart rate [44]. Furthermore, the acute infusion increased the activity of the renal, adrenal and lumbar sympathetic nerves. Shek et al. [22] recently found that leptin infusion in rats increased the arterial blood pressure and heart rate, possibly by enhancement of sympathetic nervous activity. In humans the situation seems more complex, as the plasma leptin level was not correlated with muscle sympathetic nerve activity [45] or renal spillover of noradrenaline [46]. A correlation between BMI-adjusted leptin levels and the 24-h heart rate has been demonstrated in untreated patients with hypertension [18]. In our patients, the mean resting heart rates were similar in the hypertension group and the controls, but it is possible that treatment and 24-h measurements may account for the difference. Thus a pathophysiological relationship between insulin resistance, hyperleptinaemia, obesity and hypertension seems probable from the results of the present and other studies [21,28,47–49], but human studies of the effects of leptin and its interactions with insulin and renal sodium metabolism in normotensive and hypertensive populations will be needed in order to clarify these interactions.

In conclusion, the circulating leptin concentration is greatly elevated in patients with arterial hypertension. The high plasma concentration of leptin is probably caused by increased release of leptin from abdominal (especially mesenteric and omental) and gluteal stores of adipose tissue. The kinetics of leptin in arterial hypertension require further investigation.

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