Renal clearance of endogenous leptin in hypertensive humans with or without renal artery stenosis

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Fogteloo, A. J., A. E. Meinders, H. Pijl, A. A. Kroon, M. Frölich, and P. W. De Leeuw. Renal clearance of endogenous leptin in hypertensive humans with or without renal artery stenosis. Am J Physiol Endocrinol Metab 281: E400–E404, 2001.—In humans, the kidney is involved in leptin clearance from the body. The present study was performed to assess the renal extraction of leptin in hypertensive patients with or without renal artery stenosis. Sixty-five hypertensive subjects (39 males and 26 females) underwent catheterization of the renal artery and both renal veins with blood sampling for measuring leptin levels. Blood flow to both kidneys was measured by the xenon washout technique. From these data, renal leptin uptake and renal fractional extraction of leptin were calculated. Endogenous creatinine clearance ranged from 24 to 191 ml/min in the males and from 20 to 149 ml/min in the females. In 25 patients, radiological signs of renal artery stenosis were present. Total renal leptin uptake by both kidneys averaged 141 ± 47 ng·min⁻¹·100 g⁻¹. No differences in leptin uptake were found between males and females or between patients with or without renal artery stenosis. The average renal extraction fraction of leptin was 6 ± 2%. Renal leptin uptake and renal extraction fraction of leptin did not correlate with arterial leptin concentrations or with blood pressure, endogenous creatinine clearance, or the presence or absence of renal artery stenosis. In hypertensive patients with or without renal artery stenosis, the kidney removes only a small fraction of circulating leptin from the body within one passage. This fraction remains relatively constant despite wide variations in renal function or circulating leptin.

IN ADDITION TO ITS ROLE in cardiovascular homeostasis, the kidney may contribute to intermediary metabolism by clearing from the circulation several hormones with a bearing on metabolic processes. One of these hormones is leptin, a 16-kDa polypeptide that is involved in the lipostat feedback loop to suppress food intake and to increase energy expenditure (1). Leptin concentrations are strongly correlated with body mass index (16). Leptin concentrations may also be correlated with blood pressure, but this relationship is less clear (19). It is not known whether an increase in leptin production or a decrease in leptin clearance causes higher leptin levels such as those sometimes found in hypertension. A few studies in humans have addressed the renal clearance of leptin by using measurements of the arteriovenous concentration gradient over the kidney (8, 13, 18). The results from these investigations suggest that ~80% of total body leptin clearance can be attributed to the kidney. However, these studies comprised only small numbers of patients, and in none of them were blood pressure data mentioned. This prompted us to study the renal clearance of leptin in more detail in hypertensive patients, in whom renal angiography and renal vein catheterization were indicated for workup of possible renovascular hypertension.

PATIENTS AND METHODS

Sixty-five patients (39 males and 26 females), in whom a renovascular origin of their hypertension was suspected, participated in the study. The diagnosis of hypertension was made when office systolic blood pressure was ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg on at least three different occasions. When on the basis of clinical, biochemical, or ultrasound data renovascular disease was suspected, patients underwent digital subtraction angiography with renin sampling. Patients were asked to adhere to a sodium-restricted diet (55 mmol of sodium per day) during the last week before study. Dietary compliance was checked by measuring sodium and creatinine excretion in 24-h urine, collected on the day before angiography. Inasmuch as patients were using antihypertensive agents, these drugs had been discontinued ≥3 wk before angiography.

All investigations took place in the morning between 0800 and 1200, when patients were fasting and had remained recumbent from 2200 the preceding day. Arterial and renal venous cannulation was performed, under fluoroscopic control, via the femoral route by use of the Seldinger technique. Before the injection of any contrast material, blood pressure was measured intra-arterially, and blood samples were drawn simultaneously from the renal artery and vein for determination of active renin, leptin, hematocrit (Hct), creatinine, and oxygen saturation. The latter was used to check

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whether the venous catheter had been properly positioned in the renal vein (10). Blood for leptin measurements was collected on heparin and centrifuged immediately at 2,000 g for 20 min. Plasma was then put in aliquots and stored at −80°C until assay. Immediately after blood sampling, renal blood flow (RBF) was measured in both kidneys with the xenon washout method (6). Flow was always determined first in the left kidney and subsequently in the right one.

Written informed consent was obtained from all patients, and the Medical Ethical Committee of the Maastricht University Hospital had approved the study protocol.

**Laboratory methods.** Plasma leptin concentrations were assayed with a standardized RIA (Linco Research, St. Charles, MO) with a sensitivity of 0.5 ng/ml. The intra-assay coefficients of variation varied from 6 to 7% over leptin concentrations ranging from 3 to 80 ng/ml. The interassay coefficients of variation were 10.2, 5.5, and 7.2% for concentrations of 3.9, 11.3, and 63.8 ng/ml, respectively. All samples were assayed in the same run by the same person.

Plasma creatinine was measured using the standard Jaffé technique on a Hitachi 747 analyzer (Boehringer Mannheim, Mannheim, Germany).

**Calculations.** Body mass index (BMI) was calculated as body weight (kg) × height (m)². As a measure of glomerular filtration rate, endogenous creatinine clearance was calculated from plasma creatinine with the formula of Cockcroft and Gault (2). The percentage of stenosis in the renal artery was measured with specially developed software.

RBF was determined as described previously (6). Flow data were analyzed for the right and the left kidney separately and expressed in milliliters per minute per 100 grams. Renal plasma flow (RPF) was calculated with the formula RPF = RBF × (1 − Hct). From the data of renal plasma flow and leptin concentrations, the following variables were calculated: arterial delivery of leptin (total amount of leptin entering the kidneys), venous efflux of leptin (total amount of leptin coming out of the kidneys), and the extraction of leptin. The total arterial leptin delivery to the kidneys amounts to: arterial leptin × (RPF₉ + RPF₈), where RPF₉ and RPF₈ represent renal plasma flow through the left and right kidney, respectively. Total venous efflux of leptin can be calculated as (V₉ × RPF₉) + (V₈ × RPF₈), where V₉ and V₈ represent the concentrations of leptin in the left and right renal veins, respectively. Renal uptake (RLU) by each kidney was computed as RPF × (aortic leptin concentration − renal venous leptin concentration) for that kidney and expressed in nanograms per minute per 100 grams. Total RLU was taken as the sum of the uptake by the right and the left kidney. Because kidneys do not produce leptin, total renal fractional extraction (RFEL) of leptin can be derived from the formula (total arterial delivery − total venous efflux) × (total arterial delivery)⁻¹ × 100%.

**Statistical analysis.** Results are expressed as means ± SE. For analysis, Student’s t-tests for unpaired and paired data were used where appropriate. Because leptin concentrations proved not to be normally distributed, comparisons and correlations were based on log-transformed data. Univariate and multivariate regression analyses to adjust for confounding factors were applied to assess potential relationships between variables. All calculations were performed using the SPSS program (SPSS, Chicago, IL). A P value <0.05 was considered to be statistically significant. The study had a power of 85% to detect at the 5% level (two-tailed) a difference in leptin concentrations of 5% between the renal artery and vein.

**RESULTS**

The clinical characteristics of all 65 patients are shown in Table 1. In the males, intra-arterial blood pressure ranged from 127 to 238 mmHg systolic and from 59 to 144 mmHg diastolic pressure. In the females, these figures were 113 to 262 mmHg systolic and 56 to 128 mmHg diastolic pressures, respectively. Endogenous creatinine clearance (ECC) ranged from 24 to 191 ml/min in males and from 36 to 149 ml/min in females. In 25 patients (38%), angiographic signs of renal artery stenosis were present (atherosclerosis in 22 and fibromuscular dysplasia in 3). The degree of stenosis ranged from 30% to almost complete occlusion. In eight patients with a stenosis (three males and five females), it was technically impossible to measure RBF in either one or both kidneys because of too much arterial narrowing. Moreover, in one of these eight patients, no blood could be obtained from the left renal vein. Therefore, total renal uptake and fractional excretion of leptin could be analyzed in only 57 subjects. In all of these, renal venous oxygen saturation in both veins was 90% or higher. Twenty of the 57 patients (35%) had signs of renal artery stenosis (RAS); the remainder were considered to have essential hypertension (EH). Data for arterial and renal venous leptin levels, as well as for RLU and RFEL, are given in Table 2. Both in men and in women, positive correlations were found between body weight and arterial leptin concentrations (r = 0.623, P < 0.005 for men; r = 0.664, P < 0.002 for women) and between BMI and arterial leptin (P < 0.001 for both sexes). No correlations were apparent between arterial leptin concentrations on the one hand and blood pressure, RBF, or the percentage of RAS on the other. Nor was there a correlation between BMI and blood pressure. Figure 1 illustrates the relationship between ECC and arterial leptin. In males but not in females, a positive relationship was found between ECC and arterial leptin (r =

<table>
<thead>
<tr>
<th>No.</th>
<th>Age, yr</th>
<th>Body Wt, kg</th>
<th>BMI, kg/m²</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>Cl_{creatinine}, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>65</td>
<td>54 ± 2</td>
<td>77 ± 2</td>
<td>26.7 ± 0.6</td>
<td>184 ± 4</td>
<td>97 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18–79)</td>
<td>(47–136)</td>
<td>(18–44)</td>
<td>(113–262)</td>
<td>(56–144)</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>51 ± 3</td>
<td>70 ± 2</td>
<td>26.6 ± 1.0</td>
<td>192 ± 8</td>
<td>99 ± 4</td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>56 ± 3</td>
<td>82 ± 3</td>
<td>26.8 ± 0.7</td>
<td>178 ± 5</td>
<td>96 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE with ranges in parentheses. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cl_{creatinine}, creatinine clearance.
In multivariate analysis with ECC and sex as independent variables and arterial leptin concentration as a dependent variable, the sex differences remained significant. Arterial leptin concentrations did not differ significantly between patients with EH (7.6 ± 1.0 ng/ml) and those with RAS (6.0 ± 0.9 ng/ml).

As shown in Fig. 2, plasma leptin was significantly lower in males than in females at all sampling sites. In both sexes, plasma leptin concentrations were significantly lower in the renal veins than in the renal artery (P < 0.001). Again, sex differences remained significant in multivariate analysis. In Fig. 3, the relationship between venous and arterial leptin concentrations is depicted for both kidneys separately. The regression lines describing these relationships are superimposable, with a slope (regression coefficient) of 0.8.

In the whole group, total RLU averaged 141 ± 47 ng·min⁻¹·100 g⁻¹. Although arterial delivery of leptin was numerically greater in females than in males, and also greater in patients with EH than in those with RAS (as a result of a difference in blood flow), differences were not statistically significant. RFEL amounted, on average, to 6 ± 2%. Neither the absolute arteriovenous leptin difference, nor the RLU and the RFEL, correlated with arterial leptin concentrations, blood pressure, ECC, or the degree of RAS. This was true both for the univariate analysis and after adjustment for sex, height, and weight. Moreover, differences in arterial delivery of leptin such as those found in women compared with men or in EH compared with RAS were matched by similar differences in the amounts of leptin removed by the kidney, so that RFEL was relatively constant in all subgroups of patients.

**DISCUSSION**

The main finding from the present study is that, in hypertensive patients, the kidney removes only a small fraction (<10%) of arterially delivered leptin within one passage from the circulation. In addition, our data show that this extraction is relatively independent of renal function. At least two pieces of evidence support the latter conclusion. First of all, we found that, over the concentrations studied, there was a linear relationship between arterial and renal venous leptin levels. Moreover, RLU and RFEL were not significantly affected by the presence of RAS. In other words, at least up to arterial leptin concentrations of ~30 ng/ml and over a wide range of RBF values, saturation of the renal leptin removal mechanism does not seem to occur, and extraction of the peptide remains relatively constant. Second, no relationship whatsoever was apparent between plasma creatinine or creatinine clearance and leptin extraction. This clearly illustrates that a (moderate) decline in renal function per se is not associated with accumulation of leptin in plasma. Of course, our data do not exclude the possibility of reduced leptin clearance in patients with more severe impairment of renal function than that encountered in the present study. However, even though several studies have shown that leptin levels are increased in patients with chronic renal failure (7, 12, 13, 17, 18), this is not an invariable finding (14, 15), and experiments in rats even suggest that hyperleptinemia after nephrectomy may be only transient (5, 11). These data indicate that in case of an impaired renal function or

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**Table 2. Arterial and venous leptin levels, renal leptin uptake, and fractional extraction of leptin in subjects with essential hypertension or renal artery stenosis**

<table>
<thead>
<tr>
<th></th>
<th>Essential Hypertension</th>
<th>Renal Artery Stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>L_A, ng/ml</td>
<td>7.6 ± 1.0</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>L_LV, ng/ml</td>
<td>5.9 ± 0.8</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td>L_RV, ng/ml</td>
<td>6.8 ± 0.8</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>RLU, ng·min⁻¹·100 g⁻¹</td>
<td>188 ± 65</td>
<td>58 ± 65</td>
</tr>
<tr>
<td>FE, %</td>
<td>8 ± 2</td>
<td>3 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. L_A, arterial leptin concentration; L_LV, leptin concentration in the left renal vein; L_RV, leptin concentration in the right renal vein; RLU, renal leptin uptake; FE, fractional extraction of leptin.
renal insufficiency, leptin levels can remain stable, either by a concurrent decrease in leptin production or perhaps because the kidney is not the only organ to remove leptin from the circulation, and other mechanisms involved in leptin clearance can take over when renal function is jeopardized.

Because we did not measure total body clearance of leptin, our results do not allow estimating directly the contribution of the kidney to overall removal of leptin. Nevertheless, if we assume a total renal weight of 300 g and a plasma leptin clearance of \(\frac{1.5}{2}\text{ml kg}^{-1}\text{min}^{-1}\), as suggested by Klein et al. (9), our data would indicate that the kidney contributes to total body clearance of leptin by 20% at the most. This is in sharp contrast to the observations of Meyer et al. (13), who calculated an 80% contribution of the kidney to overall leptin removal. However, these authors did not measure systemic leptin clearance either, and the high values for RPF that they presented (with an average filtration fraction below 15%)! suggest that they have overestimated kidney removal of leptin.

In the present study, we found the renal extraction of leptin to be in the order of 6%. Sharma et al. (18) found a similar extraction of \(\sim 5\%\) in 14 patients who underwent aortic and renal vein sampling. When these investigators divided their patients to show eight with a serum creatinine level \(<124\mu\text{mol/l}\) and six with higher levels, they found an RLU of 12% in the former group and no uptake in the latter. However, their conclusion that the kidney does not take up leptin with mild to moderate renal insufficiency is not justified. The serum leptin levels were actually lower in their patients with elevated serum creatinine than in those with normal creatinine levels. In our opinion, therefore, the average extraction rate of 5% derived from all 14 patients should be the figure with which our data should be compared. Jensen et al. (8) studied four patients who underwent arterial and renal venous catheterization and found in three of them small differences in plasma leptin across the kidney similar to those we found, and an unexplained big difference with a clearly elevated plasma leptin concentration in the fourth patient (8). Finally, Meyer et al. (13) studied the concentration difference of leptin in plasma obtained from one renal vein and arterialized venous blood from a dorsal hand vein in 16 normal volunteers. They found an average extraction of 13%; however, as already mentioned above, their data for RPF are not very realistic. So far, our study is the largest to report on the transrenal gradient of leptin, and on the basis of the combined data from the literature and ours, it seems fair to state that the kidney normally extracts 5–10% of arterially delivered leptin.

The renal clearance of leptin is thought to occur via glomerular filtration and renal tubular extraction and metabolism. Because the molecular weight of circulating leptin is \(\sim 16\text{kDa}\), the hormone should theoretically be able to pass the glomerular membrane completely. However, the extraction fraction of \(\sim 6\%\) is substantially less than the filtration fraction of the normal kidney. Therefore, renal removal of leptin cannot be explained only by simple glomerular filtration. Either the peptide is completely filtered and largely reabsorbed by proximal tubular cells, or glomerular filtration of this hormone is hindered as a result of its negative electric load. Because normally no leptin is found in the urine, the former explanation seems to be the more likely one, provided that leptin is not metabolized in the tubules. The mechanism of complete filtration and reabsorption could also account for our observation that a decline in renal function does not affect the fractional excretion of leptin. Indeed, with an RFEL of 6%, glomerular filtration would have to fall to very low levels before an impairment of extraction would become apparent. In some studies an inverse relationship has been found between glomerular filtration rate and plasma leptin concentrations (14), and in experimental animal models, the systemic clearance of leptin decreases by \(\pm 30\%\) after ureteral ligation (3–5). Although at first sight these data would seem to be at variance with ours, closer examination of the literature data reveals that substantial rises in leptin occur only when glomerular filtration rate falls below very low levels. Our data do not allow us to exclude the possibility that leptin production in patients with mild to moderate renal insufficiency is decreased, but it is
unlikely that this can fully account for the relatively low leptin levels observed in this and other studies. Thus, even when its function is severely impaired, the kidney may still play a role in leptin clearance.

At the moment of study, some patients had normal blood pressure, but all were known to have had hypertension for a long period. We were not able to show any correlation between blood pressure and arterial leptin levels or RLU. Although the data on the relationship between circulating leptin and blood pressure are conflicting (19), the present study does not favor the hypothesis of reduced renal clearance of this hormone in hypertensive patients. Nevertheless, an obvious limitation of our study is that we did not include a normotensive control group with comparable renal function. However, without a clinical indication to perform renal arterial and venous catheterization, the inclusion of normotensive controls would not be ethically justified.

In summary, we found in hypertensive patients that the kidney extracts ~6% of arterially delivered leptin and that this extraction is independent of the height of blood pressure or of renal function.

REFERENCES