Regional leptin kinetics in humans[1–3]

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ABSTRACT

Background: Leptin is known to be cleared by the kidney, a tissue with substantial leptin receptor mRNA expression; however, lung, liver, and muscle tissues also express leptin receptor messenger RNA and it is not known whether these tissues also clear leptin from the circulation.

Objective: This study was conducted to determine whether net leptin clearance takes place in the pulmonary, splanchnic, and leg tissue beds to a similar extent as in the kidney.

Design: Plasma leptin concentrations were measured in blood entering and exiting the renal bed, pulmonary bed, splanchnic bed, and leg in 4 groups of subjects. Regional plasma flow was measured in 3 of the 4 groups.

Results: Renal leptin uptake was substantial, whereas no net uptake of leptin by the splanchnic or pulmonary vascular beds was detected; leg tissue was a net leptin producer. Net leptin release by leg tissue, relative to leg adipose tissue mass, was comparable with that reported previously for abdominal subcutaneous adipose tissue.

Conclusion: These results confirm that the kidney is a significant site of leptin clearance in humans, whereas pulmonary and splanchnic beds are not. *Am J Clin Nutr* 1999;69:18–21.

KEY WORDS Body composition, kidney, splanchnic bed, humans, leptin, kinetics

INTRODUCTION

Leptin is a 16-kDa peptide hormone that acts in the hypothalamus to influence energy intake, energy expenditure, and hormonal function (1–4). The production and catabolism of leptin have been investigated recently. Leptin is produced solely by adipocytes in men and nonpregnant women and is secreted into the systemic circulation (1). Plasma leptin concentrations increase with increasing adiposity (5), primarily because of increased adipose tissue leptin production rather than decreased clearance (6). The kidney has been found to be an important site of leptin clearance both in rodents (7–9) and humans (10, 11). Indeed, total nephrectomy markedly decreases leptin clearance in rats (7), and chronic renal failure increases plasma leptin concentrations in humans (12). Leptin receptor messenger RNA (mRNA) is highly expressed in the kidney (13, 14), suggesting that receptor-mediated transport may be necessary for tissue uptake and catabolism. Leptin receptor mRNA is also expressed in high quantities in the lung and to a lesser extent in the splanchnic bed (liver, spleen, and small intestine) and skeletal muscle (13–19). However, the importance of nonrenal tissues in leptin clearance is not known.

The studies reported herein were conducted to evaluate in vivo leptin clearance by tissues that are known to have significant expression of leptin receptor mRNA. Arteriovenous balance techniques were used to evaluate leptin clearance by the kidneys, lungs, splanchnic bed, and legs in obese and nonobese humans.

RESEARCH DESIGN AND METHODS

Subjects

A total of 47 volunteers and 10 patients participated in these studies. There were 4 separate studies conducted as part of these investigations: *1*) 43 volunteers participated in experiments that involved the collection of femoral artery, femoral vein, and hepatic vein blood samples to examine leg and splanchnic leptin metabolism; *2*) blood samples were obtained from 6 hospitalized patients to assess whether substantial pulmonary uptake of leptin occurs; *3*) a single set of arterial and bilateral renal venous samples were obtained from 4 patients to confirm that plasma leptin concentrations are essentially equal in the right and left renal vein; and *4*) arterial and renal venous samples were collected from 4 volunteers undergoing research studies during which renal plasma flow was measured. This allowed us to examine renal leptin uptake in a more quantitative fashion.

The research volunteers (studies 1 and 4) were participants in studies of regional free fatty acid and amino acid metabolism, some of which have been published (20). All research volunteers were weight stable at the time of the study and consumed their meals in the Mayo Clinic General Clinical Research Center (GCRC) for ≥3 d before the studies began. All female research volunteers were premenopausal. The renal vein blood samples were collected from 4 patients undergoing bilateral renal vein catheterization for renal vein renin studies to evaluate possible

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renovascular hypertension. Renal blood flow was not measured in these studies; therefore, rates of leptin uptake could not be calculated. The 6 patients with pulmonary artery and arterial catheters in place for hemodynamic monitoring were selected to exclude those with pulmonary dysfunction or severe congestive heart failure. These studies were approved by the Mayo Foundation and the Washington University Institutional Review Boards.

Study protocol

All research volunteers were admitted to the Mayo GCRC the evening before the study began. Body fat was measured by using dual-energy X-ray absorptiometry (21). On the morning of the study, volunteers were transferred to the Vascular Radiology Department, where they were anesthetized for catheter placement as described previously (22). After the volunteers returned to the GCRC, they were allowed to rest for 30–60 min. Leg and splanchnic blood flow were measured by using a femoral arterial infusion of indocyanine green. A series of simultaneous arterial and venous (renal vein or hepatic and femoral vein) blood samples were obtained over 30–40 min. Plasma was separated immediately by cold centrifugation (3000 × *g*, 15 min, 4 °C) and stored at −70 °C until analyzed.

To assess the role of the kidney in clearing leptin, 4 additional volunteers underwent catheterization of the femoral artery and right renal vein in the Mayo Vascular Radiology Laboratory. On return to the Mayo GCRC, a series of 5 blood samples were obtained from the femoral artery and renal vein for measurement of plasma leptin concentrations as described above. Renal plasma flow was measured by using an intravenous infusion of 12 mg *p*-aminohippuric acid/min. The patients undergoing renal vein renin studies had a single sample of blood taken from an artery and the right and left renal veins. Replicate blood samples (2 to 3) were obtained from each site from patients with pulmonary artery catheters in place.

Analysis of samples

Plasma leptin concentrations were determined by radioimmunoassay (23) (Linco Research, St Louis). The intraassay CVs were 8.3%, 4.7%, and 3.4% at 3.8, 21.6, and 39.7 μg/L, respectively. Interassay CVs were 7.2% and 5.3% at 2.8 and 13.5 μg/L, respectively. Serial dilution of an elevated serum sample to assess linearity gave the following results: expected = 1.02 × observed − 0.07 (R^2 = 0.989). Samples were always run such that arterial and venous samples from the same subject were included in the same batch. Total-body and regional fat mass were measured by using dual energy X-ray absorptiometry (21). Plasma indocyanine green concentrations were measured on the day of the study with a spectrophotometer. The CV for repeated measures of leg and splanchnic plasma flow over 30 min in our laboratory averaged 14% and 15%, respectively. Arterial and renal venous *p*-aminohippuric acid concentrations were measured with a colorimetric method (24). The CV for repeated measures of renal plasma flow over 30 min for these subjects averaged 4%.

Calculations

Leg and splanchnic plasma flow (25, 26) and renal plasma flow (27) were calculated as described previously. Leg, splanchnic, and renal leptin uptake or release were determined by multiplying the arteriovenous differences by the regional plasma flow rates.

Statistical analysis

All values are presented as means ± SEMs, except where otherwise indicated. Differences between lean men, lean women, obese men, and obese women were assessed by using one-way analysis of variance. When there were significant differences, two-sided Student's *t* tests were performed. A nonparametric test (Mann-Whitney *U* test) was used to compare leg leptin release values in obese and lean women because the variances were significantly different. To assess whether leg leptin release was related to leg fat mass, the natural logarithm of leg leptin release was correlated with leg fat mass as measured by dual-energy X-ray absorptiometry. OXSTAT V was used for the statistical analyses (Microsoft Corp, Redmond, WA).

RESULTS

Subject characteristics

The characteristics of the 43 volunteers participating in the leg and splanchnic catheterization studies are provided in **Table 1**. Values for lean and obese men and women are presented separately. The differences in total body fat and leg fat between the different groups were as expected. Two women and 2 men [age: 30 ± 3 y; BMI (in kg/m^2): 25.2 ± 1.5; body fat: 30 ± 5%] participated in the Mayo GCRC renal venous studies. The 10 patients (8 men and 2 women) from whom blood samples were obtained for measurement of arterial and mixed venous (n = 6) or renal venous (n = 4) plasma leptin concentrations were aged 60 ± 7 y and had a mean (±SD) BMI of 27.3 ± 3.5.

Splanchnic and leg leptin kinetics

Leptin concentrations in arterial, femoral venous, and hepatic venous plasma for the 4 different groups undergoing the leg and splanchnic balance studies are provided in **Table 2**. Hepatic venous plasma leptin concentrations were not significantly different from arterial plasma leptin concentrations in any of the groups; therefore, splanchnic leptin balance was not significantly different from zero in any of the groups. Splanchnic plasma flow was 867 ± 60 and 868 ± 108 mL/min in lean and obese women and 881 ± 45 and 1025 ± 83 mL/min in lean and obese men, respectively.

Rather than finding net leptin uptake in the leg, we observed that leg tissue was a net leptin producer. Leg plasma flow in lean and obese women was 265 ± 19 and 336 ± 39 mL/min and in lean and obese men was 207 ± 19 and 332 ± 43 mL/min, respectively. Net leg leptin release in obese women was 787 ± 475 ng/min,

TABLE 1
Characteristics of subjects participating in the leg and splanchnic balance studies[1]

	Lean women (n = 12)	Lean men (n = 13)	Obese women (n = 10)	Obese men (n = 8)
Height (cm)	166 ± 2[2]	179 ± 3	163 ± 2[2]	177 ± 2
Weight (kg)[3]	63.2 ± 2.1	77.1 ± 2.4	90.0 ± 2.8	102.0 ± 4.1
BMI (kg/m^2)	22.9 ± 0.6	24.0 ± 0.5	33.8 ± 0.8	32.4 ± 1.2
Body fat (kg)[3]	19.9 ± 1.7	13.0 ± 1.1	43.8 ± 2.7	31.5 ± 2.5
Leg fat (kg)[3]	7.7 ± 0.7	3.9 ± 0.3	15.1 ± 1.5	10.1 ± 1.0

[1] $\bar{x}$ ± SEM.
[2] Significantly different from lean and obese men, $P < 0.05$.
[3] Each group was significantly different from every other group, $P < 0.05$.

TABLE 2
Plasma leptin concentrations for the subjects participating in the leg and splanchnic balance studies[1]

Leptin	Lean women (n = 12)	Lean men (n = 13)	Obese women (n = 10)	Obese men (n = 8)
Arterial	7.15 ± 0.90	2.30 ± 0.22	27.57 ± 3.73	8.48 ± 1.39
Femoral vein	8.2 ± 1.02[2]	2.73 ± 0.26[2]	29.63 ± 4.85	9.19 ± 1.32[2]
Hepatic vein	7.21 ± 0.90	2.27 ± 0.22	25.69 ± 3.60	8.61 ± 1.16

[1] $\bar{x}$ ± SEM.
[2] Significantly different from arterial, $P < 0.005$.

which was greater than the release rate in lean women, which was 264 ± 70 ng/min, but not significantly so. Leg leptin release rates in obese and nonobese men were 153 ± 29 and 103 ± 20 ng/min, respectively ($P < 0.05$, obese compared with lean). Leg leptin release relative to leg fat mass was 2.4 ± 0.5, 3.1 ± 0.8, 4.3 ± 2.8, and 2.4 ± 0.05 ng · 100 g fat^{-1} · min^{-1} in lean men, lean women, obese women, and obese men, respectively (NS between groups). There was a positive, linear correlation between leg fat mass and the natural logarithm of leg leptin release (**Figure 1**).

Pulmonary leptin kinetics

Cardiac output in the patients in whom pulmonary arterial (mixed venous) and arterial leptin concentrations were measured was 6.6 ± 1.0 L/min. Mixed venous and arterial leptin concentrations were 9.1 ± 3.4 and 9.1 ± 3.2 μg/L, respectively. The mean (±SD) absolute difference between arterial and mixed venous plasma leptin concentrations was 0.25 ± 0.27 μg/L, indicating consistent, good agreement between leptin concentrations in arterial and mixed venous blood.

Renal leptin kinetics

The mean arterial leptin concentration was 30.2 μg/L in the patients undergoing renal vein catheterization studies for clinical reasons. The arterial and right and left renal venous leptin concentrations are provided in **Table 3**. The difference in arteriorenal venous leptin concentrations averaged 5.9 μg/L (range: 0.4–21.2 μg/L). The right and left renal venous leptin concentrations were similar in all subjects. Arterial and renal venous plasma leptin concentrations in volunteers undergoing the renal vein catheterization studies in the GCRC were 8.5 ± 2.2 and 7.1 ± 2.2 μg/L, respectively, with an arteriovenous difference of 1.4 ± 0.6 μg/L. Renal plasma flow was 778 ± 124 mL/min, resulting in a net renal leptin clearance rate of 1316 ± 744 ng/min.

DISCUSSION

These studies were undertaken to examine the roles of the pulmonary, splanchnic, renal, and leg tissue beds as sites of leptin clearance, leptin production, or both. Leptin concentrations were measured in arterial and venous plasma samples taken from volunteers undergoing free fatty acid and amino acid studies and from patients with vascular catheters in their pulmonary arteries and renal veins for clinical purposes. We confirmed the importance of the kidney as a site of leptin clearance, but found no evidence of significant leptin clearance by the pulmonary or splanchnic beds. The net release of leptin from leg tissue, when expressed relative to leg fat mass, was at rates remarkably similar to those observed from abdominal subcutaneous fat (6).

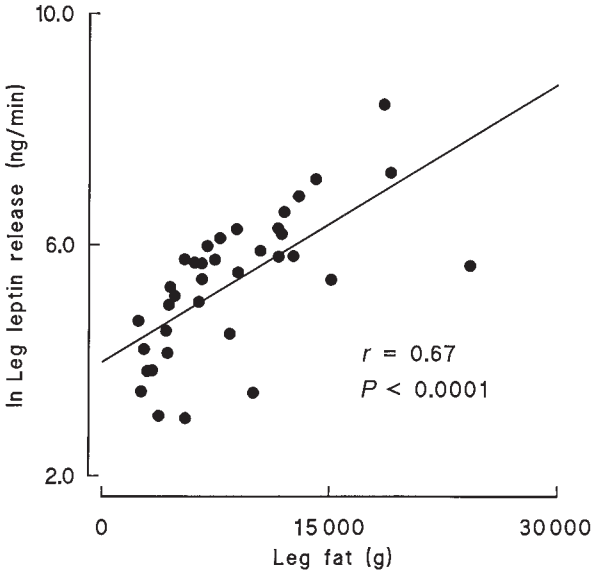

FIGURE 1. The natural logarithm (ln) of leg leptin release plotted against total leg fat mass (as measured by dual energy X-ray absorptiometry) for the volunteers participating in the leg leptin balance studies.

We were unable to detect net clearance of leptin by splanchnic tissues; however, 2 factors limited our ability to conclude that splanchnic tissues do not take up leptin. First, visceral adipose tissue leptin release could offset some splanchnic leptin clearance. For example, visceral adipose tissue was likely ≈2–4 kg (28) in the obese men participating in the splanchnic balance studies, an amount that could release 6–12 μg leptin/min into the portal vein if visceral fat releases leptin at rates comparable with abdominal subcutaneous fat (6). Splanchnic tissues in obese men could therefore take up this amount of leptin and yet no net splanchnic leptin uptake would be detectable. The amount of leptin released from visceral adipose tissue in lean men and lean and obese women should be even less than that estimated for obese men and, therefore, should be less of a confounding factor in evaluating splanchnic leptin clearance. The second limiting factor is the high rate of splanchnic blood flow, which tends to narrow the differences between arterial and venous leptin concentrations for a given rate of leptin uptake (or release). This makes it more difficult to accurately assess low rates of leptin uptake, although the collection of multiple arterial and hepatic venous blood samples in each volunteer should increase the likelihood of detecting small differences. We cannot exclude the possibility that small amounts of leptin are produced or cleared by the splanchnic bed; however, if net splanchnic leptin uptake does occur, it is minimal compared with that of the kidney.

TABLE 3
Bilateral renal venous plasma leptin concentrations for patients undergoing renal vein catheterization for clinical purposes

Subject	Arterial	Right renal vein	Left renal vein
		μg/L	
1	18.8	17.8	17.7
2	84.2	60.3	65.7
3	10.8	9.7	10.0
4	7.1	6.8	6.7

In mice, leptin receptor mRNA expression in the lung is substantially greater than that in the kidney (13). We found no evidence of pulmonary leptin clearance in our subjects, which suggests that leptin clearance may not be proportional to leptin receptor expression. Plasma leptin concentrations were virtually identical in the arterial and mixed venous (pulmonary artery) samples. Analogous with the splanchnic bed, however, high rates of pulmonary blood flow increase the difficulty in detecting leptin uptake unless the lung is a major site of clearance (equal to or greater than that by the kidney).

Data from the present study lend further support to the notion that the kidneys are the main site for plasma leptin clearance in humans. The rate of renal leptin uptake in our subjects was similar to that reported by Meyer et al (10). Studies performed in mice (9) and humans (10) found little or no intact leptin in urine, suggesting that renal leptin clearance involves active degradation by renal parenchyma and not simple urinary excretion. Of interest, renal leptin clearance is not decreased in animal models with defective leptin receptors (7). This does not exclude the existence of a receptor-mediated leptin clearance process, however, because leptin binding to the defective receptor is known to occur (13). Most patients with end-stage renal disease who are obese have elevated plasma leptin concentrations, whereas most of those who are lean have normal plasma concentrations (12). These observations suggest that normal plasma leptin concentrations can be maintained, despite end-stage renal disease, if the rate of leptin production is kept low, presumably by decreasing leptin production or by ensuring adequate leptin clearance by the remaining renal parenchyma or other tissues. However, higher rates of leptin production can overwhelm these regulatory mechanisms and generate increased plasma leptin concentrations.

We estimated leg adipose tissue leptin release rates by measuring differences between arterial and venous plasma leptin concentrations and relating net release rates to leg fat mass. If leptin is cleared by leg muscle, however, leg adipose leptin release would be greater than we predicted. Of note, the values we obtained were remarkably similar to those reported previously for subcutaneous abdominal adipose tissue (6). This suggests that leptin release in leg adipose tissue is equal to or slightly greater than abdominal subcutaneous release. These data may indicate that leptin release from different adipose tissue beds is relatively equal within a given individual.

In summary, the results of the present study further support the importance of the kidney as the major site of leptin clearance in humans. We found no evidence of significant pulmonary or splanchnic leptin uptake. In addition, there was no net leptin uptake by the leg, and the calculated rates of leg adipose tissue leptin production were comparable with those reported previously for abdominal subcutaneous adipose tissue.

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