Whole body leptin kinetics and renal metabolism in vivo

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Zeng, Jianbo, Bruce W. Patterson, Samuel Klein, Daniel R. Martin, Samuel Dagogo-Jack, Wendy M. Kohrt, Steven B. Miller, and Michael Landt. Whole body leptin kinetics and renal metabolism in vivo. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1102–E1106, 1997.—Leptin metabolism was investigated in male Sprague-Dawley rats by use of 125I-labeled leptin plasma kinetic and arteriovenous balance studies. When conscious rats received bolus venous injections of 125I-leptin, intact (precipitable) leptin quickly disappeared from circulation in a biexponential manner during the 2-h experimental period. After substantial delay, most of the injected radioactivity appeared in the urine. The data were described by a two-compartment model, which postulated that plasma leptin exchanged with a nonplasma tissue rather than glomerular filtration is the predominant elimination mechanism.

Leptin, the 16-kDa protein product of the obese gene, is an important regulator of energy balance and hypothalamic-pituitary-gonadal function. A defect in adipose tissue leptin production is responsible for obesity in obese (ob/ob) mice (3, 9, 18, 26), whereas a mutation in the hypothalamic leptin receptor is responsible for obesity in diabetic (db/db) mice (6) and fatty (fa/ fa) (19) and obese hypertensive K0letsky (23) rats. Plasma leptin concentrations affect puberty (5), reproductive function (4), and the neuroendocrine response to fasting (1).

Although there has been an explosion of research evaluating the physiological effects of leptin in the past two years, little is known about the metabolism of leptin itself. Leptin is produced exclusively by adipose tissue (26), and its rates of production and plasma concentrations are directly related to body fat mass (12). Leptin receptors are expressed in brain, lung, skeletal muscle, and kidney (24), which may be the organ most responsible for leptin catabolism. Total nephrectomy markedly decreases leptin clearance in rats (8), and chronic renal failure increases plasma leptin concentrations in humans (16). However, many details regarding leptin plasma kinetics and degradation pathways are still unknown.

The purpose of the present study was to perform a comprehensive evaluation of plasma leptin metabolism, including leptin production, catabolism, exchange with nonplasma space, and relative pool sizes. A mathematical model was used to determine leptin kinetic parameters after a bolus intravenous injection of 125I-labeled leptin in lean adult rats during steady-state conditions. In addition, leptin clearance across the kidneys was estimated in vivo by measuring arterial and renal vein plasma leptin concentrations.

METHODS

Animals

Male Sprague-Dawley rats, 375–465 g, were obtained from Harlan Bioproducts (Indianapolis, IN). All animals were fed normal rat chow and water ad libitum before being studied. Animal studies were conducted in accordance with a protocol approved by the Animal Studies Committee of Washington University.

Materials

Preparations of rat 125I-leptin suitable for injection (Linco Research, St. Charles, MO) were prepared immediately before use by adding 1.1 ml of 0.01% Triton X-100–0.23% bovine serum albumin–150 mmol NaCl to vials containing 2.3 µCi of labeled leptin.

Study Protocols

Leptin kinetic studies. Rats were anesthetized with ether and prepared for study as described by Miller et al. (17). Ureteric production was diverted to a polyethylene catheter (Intramedic PE-50, Becton-Dickinson, Sparks, MD) by ligature of the catheter (with ligature of the bladder) into an incision of the bladder. Polyethylene catheters were inserted into the femoral vein and artery, which were kept patent until analysis.

Arterial blood samples (300 µl) were obtained at 2, 4, 8, 16, 32, 60, 90, and 120 min after 125I-leptin injection. Blood samples were collected in heparinized capillary blood tubes and centrifuged immediately at 8,000 g for 1 min. The plasma supernatant was transferred to microcentrifuge tubes and stored at −20°C until analysis.

Leptin renal clearance studies. Rats were anesthetized by injection of pentobarbital sodium intraperitoneally, which
rendered them unconscious throughout the experiment. Polyethylene catheters were inserted into a femoral vein and artery. Catheters were kept patent by normal saline infusion or by filling the catheter with heparinized saline. In one group of animals renal metabolism of leptin tracer was determined. A bolus injection of $^{125}$I-leptin (0.5 ml containing 3.5 ng leptin and $1 \times 10^6$ cpm) was delivered into the femoral vein, and arterial and right and left renal vein blood samples (700 ml) were obtained simultaneously 5 min later. Blood samples were placed in heparinized capillary tubes and centrifuged immediately at 8,000 g; the plasma was stored at $-20^\circ$C until analysis. In another group of animals, endogenous arteriovenous leptin balance was determined. In these animals, $^{125}$I-leptin was not infused. Arterial, left renal vein, and right renal vein blood samples (700 µl) were obtained to determine plasma creatinine and leptin concentrations. Blood samples were placed in heparinized capillary tubes and centrifuged immediately at 8,000 g; the plasma was stored at $-20^\circ$C until analysis.

Analyses

Body fat mass. Rats killed after the leptin kinetic and renal clearance experiments just described were frozen for later analysis of body fat content. After rats were defrosted overnight, total body fat mass was measured by dual-energy X-ray absorptiometry with a Hologic QDR-1000W instrument (Hologic, Waltham, MA) (11).

Plasma leptin, creatinine, and total protein concentration. Plasma leptin concentration was measured by radioimmunoassay (Linco Research, St. Charles, MO) according to the manufacturer's protocol. The interassay coefficients of variation ranged from 3.0 to 5.7% over a concentration range of 1.6–13.6 µg/l. Creatinine and total protein were measured with a Vitros 250 analyzer (Johnson and Johnson Diagnostics, Rochester, NY).

Specific activity. Plasma proteins in 100-µl aliquots were precipitated by addition of 10 µl of 100% (wt/vol) trichloroacetic acid (TCA). The precipitates were separated by centrifugation, and radioactivity of intact leptin in the pellets was counted with a gamma counter (model 10/600, Titertek Instruments, Huntsville, AL). Supernatants (nonprecipitable fraction), considered to be degraded leptin, were also counted. Radioactivity content of urine samples was measured by counting the entire urine specimen.

Mathematical model. Compartmental modeling analysis of the data was conducted using the SAAM II program (SAAM Institute, Seattle, WA). It was assumed that $^{125}$I-leptin metabolism represented true endogenous leptin metabolism. Leptin production rate was considered to equal pool size (plasma concentration $\times$ plasma volume) $\times$ fractional catabolic rate (FCR). Plasma leptin half-life was calculated as $\ln(2)/$FCR.

Statistical Analysis

All results are presented as means $\pm$ SD. A simple linear regression was used to determine the relationship between variables, and the statistical significance of the relationships was evaluated by Spearman’s rank-sum correlation ($r_s$). Differences between arterial and venous plasma concentrations were evaluated using Student's t-test for paired samples. A P value of <0.05 was considered to be statistically significant.

RESULTS

Plasma Leptin Concentrations

Basal arterial plasma leptin concentrations were measured in 13 adult Sprague-Dawley rats. Mean leptin concentration was $2.2 \pm 0.7$ µg/l (range 1.0–5.6 µg/l). Plasma leptin concentrations correlated directly with percent body fat ($r_s = 0.84$, P < 0.0003; Fig. 1) and total fat mass ($r_s = 0.89$, P < 0.0001).

Plasma Leptin Kinetics

The kinetics of a bolus injection of radioactive tracer ($^{125}$I-leptin) was monitored over a 2-h period in five rats. Intact (precipitable) leptin decreased rapidly in a biexponential manner (Fig. 2). Plasma radioactivity in the supernatant fraction after TCA precipitation was negligible immediately after $^{125}$I-leptin injection but appeared as intact leptin disappeared (Fig. 3). Measurable radioactivity did not appear in urine until more than 15 min after the bolus injection (Fig. 4). Subsequently, most of the injected radioactivity appeared in the urine. Plasma total protein concentration was similar at the start (45 ± 3 g/l) and finish (49 ± 9 g/l) of the experimental period (n = 4; P = 0.30).

A compartmental model was developed to account for the tracer kinetics in plasma (both precipitable and nonprecipitable counts) and urine (Fig. 5). The model...
postulates that leptin exchanges between the sampled plasma compartment (compartment P) and a non-sampled, nonplasma compartment (compartment NP). After metabolic processing delays, the tracer cleared from plasma appears either directly in urine or in the plasma-nonprecipitable supernatant fraction. It was assumed that the plasma-nonprecipitable supernatant fraction, which probably includes iodotyrosine, was cleared to urine. The model provided excellent fits for the intact plasma leptin tracer (Fig. 2), the plasma supernatant fraction (Fig. 3), and urine (Fig. 4). The two clearance pathways (nonprecipitable plasma and urine), quantitatively accounted for all leptin tracer lost from plasma during the 2-h study period. An alternative model, postulating that all urine radioactivity arose from degraded leptin appearing in plasma (the anticipated mechanism if leptin was primarily eliminated by glomerular filtration or hepatic uptake), provided a poorer fit to the experimental data (Fig. 4). Other models that postulated alternative pathways of leptin degradation yielded similarly poor fits with the experimental data (not shown).

The FCR, which represented the sum of irreversible losses for both pathways of tracer leptin radioactivity, was $0.0788 \pm 0.0186$ pools/min, and plasma leptin half-life was $9.4 \pm 3.0$ min. During the 2-h study period, plasma leptin concentrations were in steady state and remained constant. Therefore, the rate of leptin elimination must equal the rate of leptin production. On the basis of body fat measurements, the rate of leptin production was $3.6 \pm 1.2$ ng·100 g fat $^{-1}$·min $^{-1}$. The ratio of forward (P to NP) to backward (NP to P) exchange constants was 3.6, suggesting that the nonplasma leptin pool was 3.6-fold larger than that of plasma. Of the radioactivity lost from intact leptin, 86.1 $\pm$ 5.8% was present in urine and 13.9 $\pm$ 5.8% was present in the plasma supernatant fraction. The turnover rate of plasma supernatant fraction (unprecipitable radioactivity generation rate) was $0.0086 \pm 0.0077$ pools/min, which was 10-fold slower than intact leptin. The delay in radioactivity appearance in plasma supernatant and in urine was 12.3 $\pm$ 4.1 and 56.4 $\pm$ 10.8 min, respectively.

**Leptin Renal Clearance**

Tracer and tracee arteriovenous balance measurements across the kidneys demonstrated net renal uptake of leptin. Left renal vein $^{125}$I-leptin tracer content was $21 \pm 1.5$% lower than arterial tracer content ($P < 0.0001$). Mean plasma leptin concentrations in the left renal vein ($2.0 \pm 0.4$ μg/l) and right renal vein ($2.1 \pm 0.4$ μg/l) were $21 \pm 8$% ($P = 0.006$) and $18 \pm 12$% ($P = 0.02$) lower, respectively, than arterial plasma leptin ($2.6 \pm 0.5$ μg/l) ($n = 5$). Mean plasma creatinine concentrations in the left ($2.6 \pm 0.1$ μg/l) and right ($2.7 \pm 0.3$ μg/l) renal veins were lower than the mean arterial plasma.
concentration (3.4 ± 0.3 µg/l), demonstrating adequate sampling of the veins.

DISCUSSION

In the present study, we used a combination of tracer kinetics and arteriovenous balance methodology to evaluate plasma leptin metabolism during steady-state conditions in normal rats. Our data demonstrate that leptin exchanges between a plasma and a nonplasma compartment, has a short plasma half-life, and is efficiently cleared from plasma by renal extraction. The majority of radioactive leptin cleared from plasma (86%) was accounted for in urine, and kinetic modeling suggested that leptin degradation was predominantly a renal process. The results of the present study strengthen the notion that the kidneys are of considerable importance in leptin metabolism. Our data are consistent with those reported by Cumin et al. (8), who found that bilateral nephrectomy in rats decreased plasma leptin clearance by 81%. In addition, we have recently found that adequate renal function is also necessary for normal leptin catabolism in humans; patients with end-stage renal failure have increased plasma leptin concentrations (16).

Our model of plasma leptin kinetics represents the least complex explanation that accounts for all the observed data. The model includes two compartments because of the biexponential loss of intact leptin from circulation. The first compartment for leptin distribution is plasma, whereas the other compartment presumably represents exchange with tissues and/or extravascular space. It is also possible that additional compartments exchange with plasma but were not identified by our model because exchange with these compartments is either faster or slower than can be resolved by a 2- to 120-min experimental protocol. Several variations of this model were tested and rejected because they did not adequately fit the data. For example, the hypothesis that all of the urine tracer derived from the plasma supernatant pool was tested and rejected; this finding suggests that urine radioactivity did not arise by simple glomerular filtration of degradation product in plasma, which could arise through degradation at a site such as liver or through reabsorption in the proximal tubule. A pathway for loss from the nonplasma compartment to either the plasma supernatant pool or urine was tested and rejected. Also, it was not possible to postulate the existence of a common degradation process from which both the plasma supernatant and urinary radioactivity were derived.

The cascade of events responsible for leptin catabolism by the kidneys is unknown. The failure of 125I-leptin to appear immediately in urine after bolus injection is inconsistent with the hypothesis that leptin is eliminated by simple glomerular filtration alone. The results of these kinetic studies suggest that renal metabolism of leptin could involve active uptake of leptin by renal tissues. The study of Cumin et al. (8) concluded that glomerular filtration was the important mechanism for renal elimination of leptin, but their studies involved administration of large amounts of exogenous leptin; use of such large amounts may have saturated uptake in the kidney. Saturation of specific uptake would prolong the half-life of leptin and enhance other pathways of elimination, such as glomerular filtration. The leptin receptor, OB-R, is abundantly expressed in the kidney. The leptin receptor gene produces multiple splice variants, and different OB-R forms are expressed by different tissues (6). The kidney expresses the short form of OB-R (24, 25), which could be involved in receptor-mediated uptake by renal tissue. These receptors have been shown to bind leptin with high affinity and specificity (20). Leptin circulates in both free (16.7 kDa) and protein-bound (>70 kDa) forms in plasma, and in lean animals leptin is predominantly protein bound (10, 22). Molecular size should prevent bound leptin from being filtered by the glomerulus. It is possible that the portion of plasma leptin in free form is filtered by the glomerulus and subsequently degraded by renal tissue. Indeed, Cumin et al. found that ureteral ligation, which affects glomerular filtration, decreased endogenous leptin clearance by 30%.

Our data demonstrate that there are many similarities in leptin metabolism between rodents and humans. The direct relationship between adiposity and plasma leptin concentration observed in our rat model, which had not previously been reported, is consistent with observations made in humans (7, 15). Mean plasma leptin half-life in rats (~9 min) was similar to values observed in humans (~25 min) (12). Furthermore, mean estimated adipose tissue leptin production rate in the present study (3.6 ± 1.2 ng·100 g fat⁻¹·min⁻¹) was similar to estimates made in humans (3.2 ± 0.5 ng·100 g fat⁻¹·min⁻¹) (12). An important issue for future study is to determine whether changes in leptin renal elimination accompany the accumulation of leptin with increasing adiposity. Because leptin concentrations change more acutely (<24 h) in response to fasting (2, 13), with extreme exercise (14) and with a diurnal pattern (21), it is possible that reduction of elimination may play a role in these day-to-day variations in plasma leptin concentrations. Studies in rats offer a convenient model to determine the mechanistic basis for these day-to-day variations.

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