Plasma leptin concentrations are only transiently increased in nephrectomized rats

MICHAEL LANDT, DANIEL R. MARTIN, JIANBO ZENG, STEVEN B. MILLER, WENDY M. KOHRT, AND BRUCE W. PATTERSON

Departments of Pediatrics, Internal Medicine, and Pathology, Washington University School of Medicine, St. Louis, Missouri, 63110

Landt, Michael, Daniel R. Martin, Jianbo Zeng, Steven B. Miller, Wendy M. Kohrt, and Bruce W. Patterson. Plasma leptin concentrations are only transiently increased in nephrectomized rats. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E495–E499, 1998.—Leptin is an adipocyte-secreted hormone that has effects on appetite and energy expenditure. Several studies have shown that end-stage renal disease results in elevated plasma leptin concentrations and that the kidney is responsible for most of leptin elimination in rodents. Leptin metabolism was investigated in rats that underwent unilateral nephrectomy to experimentally limit renal elimination function. Within 4 h of nephrectomy, plasma leptin concentrations increased from 2.9 ± 0.8 to 5.8 ± 1.0 µg/l but thereafter rapidly (<24 h) decreased to prenephrectomy concentrations, despite continued elevated plasma creatinine levels. Sham-operated rats maintained presurgical concentrations of leptin and creatinine throughout the experiment. Kinetic studies of 125I-labeled leptin elimination showed that fractional catabolic rates and half-lives of leptin in circulation were similar at 48 h in nephrectomized and sham-operated rats, suggesting that production of leptin was unchanged after nephrectomy. Excretion of 125I derived from leptin in urine of nephrectomized rats was similar to that of sham-operated rats, and residual radioactivity was increased in the remaining kidneys excised from nephrectomized rats. These results demonstrate that 1) leptin concentrations are quickly restored to presurgical levels in nephrectomized rats, and 2) it is leptin elimination, not leptin production, that compensates to maintain leptin concentrations. Rapid metabolic adaptation of remaining renal tissue may explain the restoration of normal leptin elimination in nephrectomized rats.

Increasing evidence suggests that the kidney is the principle organ responsible for elimination of circulating leptin. Arteriovenous balance studies in rodents and humans showed that the kidney efficiently extracted leptin from the renal circulation (22, 27). Removal of both kidneys or ligation of the ureters of rats decreased clearance of leptin, and whole body kinetic studies concluded that the kidney was responsible for nearly all leptin elimination (6, 7, 22). Several studies have reported elevated plasma leptin concentrations in chronic renal failure and end-stage renal disease (ESRD) patients (8, 11, 12, 18, 22, 26, 27). The underlying mechanism for the observed elevations is a matter of controversy. The lack of correlation of leptin elevations with the degree of renal impairment, as evidenced by such parameters as creatinine clearance rates, suggests that simple accumulation due to lack of elimination is not the principal cause. In some studies, most of the ESRD patients had normal leptin concentrations, after adipsy, despite near-total or total lack of functioning renal capacity, had been taken into account (18, 27).

If renal metabolism of leptin is the overwhelming means of elimination of circulating leptin, it is not clear how patients with ESRD can have normal leptin concentrations. We reasoned that either leptin production must decline to compensate for the loss of renal elimination capacity, or an alternative elimination mechanism must be activated. In either case, we reasoned that the loss of renal elimination capacity must be sensed by the organism, and some means of restoring plasma leptin concentrations must be present if normal plasma leptin concentrations are maintained in ESRD. To test this hypothesis, we experimentally reduced functional renal capacity in rats by unilateral nephrectomy, determined the time course of plasma leptin changes, and studied leptin whole body kinetics using 125I-labeled leptin.

METHODS

Materials. Male Sprague-Dawley rats, 400–450 g, were obtained from Harlan Bioproducts (Indianapolis, IN). All animals were fed normal rat chow and water ad libitum throughout these studies. Animal studies were conducted in accordance with a protocol approved by the Animal Studies Committee of Washington University. Preparations of rat 125I-leptin suitable for injection (Linco Research, St. Charles, MO) were prepared immediately before use by adding 1.1 ml of 1.0% BSA and 150 mmol/l NaCl to vials containing 2.3 µCi of labeled leptin.

Study protocols. Rats were given ether anesthesia, blood was drawn by syringe from the tail vein, and the right kidneys were removed by retroperitoneal incision. For control animals, the incision was performed, but the kidney was not...
removed. The surgical wound was closed in two layers with 6-0 prolene and staples. The time course of changes in plasma leptin was followed by serial draws of 400 µl of blood into heparin-containing microfuge tubes from the tail vein, while animals were under ether anesthesia, at 2, 4, 8, 24, and 48 h. Animals were returned to their normal environment between blood draws and allowed ad libitum access to food and water.

For the leptin kinetic studies, rats with unilateral nephrectomies or controls (sham operated) were anesthetized with ether and prepared for study as previously described (27). Urine production was diverted to a polyethylene catheter with ligature of the bladder. Polyethylene catheters were inserted into the femoral vein and artery; these were kept patent by infusion with normal saline at 4.0 ml/h using a Harvard syringe pump (femoral vein) or by filling the catheter with heparinized saline (femoral artery). After catheterization, ether anesthesia was withdrawn, and the rats were conscious during the remainder of the experiment. After a 60-min stabilization period, three 20-min urine collections and midcollection arterial blood samples (500 µl) were obtained sequentially for determination of creatinine clearance and plasma leptin concentrations. Bolus injection of 125I-leptin (0.5 ml containing 3.5 ng of leptin and 1 × 106 counts/min) was delivered via femoral vein, followed immediately by saline infusion (4 ml/h) for 96 min. Urine was collected continuously in 30-min fractions during this period. Arterial blood samples (300 µl) were obtained, at 3, 6, 12, 24, 48, and 96 min post-125I-leptin injection, into heparinized microfuge tubes. After centrifugation, plasma proteins including intact leptin were precipitated with trichloroacetic acid. The precipitated radioactivity, supernatant radioactivity, and urine radioactivity were determined as previously described (27).

Analyses. Body fat mass was determined in animals used in the above experiments by dual-energy X-ray absorptiometry with a Hologic QDR-1000 instrument (Hologic, Waltham, MA) as previously described (27). Plasma leptin was measured by radioimmunoassay (Linco Research); this assay has been extensively evaluated (16). Creatinine was measured with a Vitros 250 analyzer (Johnson and Johnson Diagnostics, Rochester, NY).

Statistical analyses. Compartmental modeling analysis of the data was conducted using the SAAM II program (SAAM Institute, Seattle, WA). It was assumed that 125I-leptin metabolism represented true endogenous leptin metabolism. Leptin production rate was considered to equal pool size (plasma concentration × plasma volume) × fractional catabolic rate. All results are presented as means ± SD. Differences between means of leptin and creatinine concentrations were evaluated for significance with Student's t-test, with P < 0.05 as significant.

RESULTS

The initial experiment followed changes in plasma leptin concentration for 48 h after unilateral nephrectomy of rats. Sham-operated rats served as controls. Plasma leptin concentrations were 2.9 ± 0.8 µg/l just before surgery in the rats that underwent unilateral nephrectomy (n = 6), which was similar to the presurgical concentration in the sham-operated rats (3.2 ± 0.8 µg/l; P = 0.50, n = 7). Leptin concentrations rose rapidly in the nephrectomized rats to reach a peak at 4 h (5.8 ± 1.0 µg/l; P = 0.0008 vs. presurgical mean) but declined thereafter to near presurgical levels by 24 h (3.1 ± 0.4 µg/l) and remained at baseline through 48 h after surgery (Fig. 1). The initial doubling of plasma leptin concentrations was consistent with the loss of one-half of elimination capacity, assuming that elimination was largely or entirely a renal function. Sham-operated rats maintained baseline levels throughout the 48-h period (Fig. 1), and leptin concentrations in sham-operated and nephrectomized rats were similar at 48 h (P = 0.73).

These results were interpreted to suggest that nephrectomized rats maintained their plasma leptin concentrations acutely. The restoration of presurgical leptin concentrations was not due to adaptation of glomerular filtration capacity, since plasma creatinine concentrations remained elevated over baseline levels and in comparison with sham-operated controls throughout the 48-h period (Fig. 1) and creatinine clearance measured at 48 h was approximately one-half in the nephrectomized rats compared with the sham-operated animals (0.46 ± 0.04 (n = 3) vs. 0.84 ± 0.18 ml·min⁻¹·100 g body wt⁻¹ (n = 4), respectively). Body fat contents of sham-operated and nephrectomized rats 48 h after surgery were similar (12.0 ± 1.6 vs. 12.1 ± 0.6%, respectively; P = 0.887), so the return to baseline leptin concentrations in nephrectomized rats at 24–48 h was not due to an acute loss of body fat.

To determine whether the observed return of plasma leptin concentrations by 24 h in the nephrectomized
had significantly more (not shown). The left kidneys of nephrectomized rats had radioactivities of liver, heart, and spleen similar (data not shown). The half-life of leptin was studied in both nephrectomized and sham-operated rats (Table 1). If production rate of leptin had been decreased in nephrectomized rats to restore baseline concentrations, the half-life of leptin would have had to increase roughly twofold to explain the observed similarity of plasma leptin concentrations to those of sham-operated rats.

In an effort to identify sites of alternative elimination of leptin that might be activated in nephrectomized rats, several organs were removed from the rats at the end of the kinetic studies, after the animals were killed, for gross measurement of associated radioactivity (Table 2). Although liver had considerable radioactivity associated with it, there was no difference in associated radioactivity between nephrectomized and sham-operated rats. The hearts and spleens from the two groups of rats also had similar amounts of radioactivity associated with these tissues. Expressing the results in relation to the gross weight of tissue made the relative radioactivities of liver, heart, and spleen similar (data not shown). The left kidneys of nephrectomized rats had significantly more (P = 0.014) associated radioactivity compared with the left kidneys of sham-operated rats (1.02 ± 0.10% of injected dose vs. 0.68 ± 0.05%, respectively) (Table 2). The distinction was somewhat reduced when the results were expressed on the basis of weight of kidney tissue, presumably because the remaining kidney in unilaterally nephrectomized rats had begun the hypertrophic process of compensating for lost renal function (data not shown). Nevertheless, this result suggests that the remaining kidney in nephrectomized rats increased metabolism of leptin rapidly, much faster than the increase in glomerular filtration that occurs over a period of weeks in nephrectomized rats (13), and thereby was responsible for the increased elimination capacity that restored plasma leptin concentrations to presurgical levels. The amounts of radioactivity excreted in urine by the same rats during the kinetic studies were similar in nephrectomized rats (expressed as the percentage of injected dose) and in sham-operated controls (9.4 ± 5.5% vs. 10.2 ± 5.5%, respectively; P = 0.848), which may reflect the ability of the remaining kidney in the nephrectomized rats to metabolize (and then excrete) as much leptin as both kidneys in the sham-operated rats.

**DISCUSSION**

The results of this study demonstrate that sudden loss of leptin elimination capacity (unilateral nephrectomy) results in only a transient increase in plasma leptin and that homeostatic mechanisms quickly (within 24 h) restore circulating leptin concentrations to presurgical levels. Only the variations seen with fasting/feeding (1, 14, 25) and inflammation/cytokine activation (9, 20) suggested that leptin concentrations might be altered acutely in physiological response to stress or infection. The observations made here raise the possibility that organisms possess a mechanism to sense acute changes in circulating leptin concentrations, such as those occurring with nephrectomy, and react to maintain leptin homeostasis.

Kinetic studies of leptin metabolism in nephrectomized rats indicate that reestablishment of plasma leptin concentrations to presurgical levels is not accomplished by reduction in production rate but instead likely involves activation or greatly increased activity of alternative (or residual) elimination capac-

### Table 1. Leptin kinetics in unilaterally nephrectomized and sham-operated rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nephrectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin half-life, min</td>
<td>19.1 ± 4.2</td>
<td>21.1 ± 10.1*</td>
</tr>
<tr>
<td>Fractional catabolic rate, pools/min</td>
<td>0.038 ± 0.007</td>
<td>0.038 ± 0.013t</td>
</tr>
<tr>
<td>% of Fractional catabolic rate of paired control</td>
<td>99 ± 30</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. *P = 0.693, †P = 1.000 vs. control rats.

### Table 2. Residual radioactivity in various organs after bolus injection of 125I-labeled leptin

<table>
<thead>
<tr>
<th>Organ</th>
<th>Sham-Operated Rats, % of Injected Dose</th>
<th>Nephrectomized Rats, % of Injected Dose</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>0.68 ± 0.05</td>
<td>1.02 ± 0.10</td>
<td>0.014</td>
</tr>
<tr>
<td>Liver</td>
<td>4.18 ± 0.34</td>
<td>4.50 ± 0.54</td>
<td>0.424</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.29 ± 0.10</td>
<td>0.45 ± 0.16</td>
<td>0.121</td>
</tr>
<tr>
<td>Heart</td>
<td>0.47 ± 0.22</td>
<td>0.53 ± 0.40</td>
<td>0.828</td>
</tr>
</tbody>
</table>

Values are means ± SD.
ity. Very little is known about the mechanism that takes up and metabolizes plasma leptin other than that it is located primarily in the kidney of normal rats and humans (6, 7, 27). There is some evidence suggesting that a functional leptin receptor may not be essential for leptin elimination; rodent genetic models of obesity with lesions of the leptin receptor, such as the db/db mouse and fa/ fa rat, have plasma leptin concentrations much higher than lean wild-type animals, but log-transformed plasma concentrations are similar to wild-type obese rodents after body fat content has been taken into account (17).

Preliminary evidence from the present studies of residual radioactivity of various organs in nephrectomized rats after bolus injection of 125I-leptin and studies of the excretion of radioactivity in the same rats suggest that adaptive changes in the metabolic capacity of the remaining kidney may be responsible for the reestablishment of prenephrectomy leptin concentrations observed 24 h after surgery. Metabolic adaptation to increase leptin elimination, if it occurs, seems much faster than other adaptations that are known to occur after unilateral nephrectomy, which require hypertrophy of the remaining kidney to generate the tissue mass, which restores functions such as glomerular filtration rate. Such hypertrophy takes weeks rather than hours to reach full effect and can be readily appreciated by a return of creatinine clearance and creatinine concentrations to near-prenephrectomy levels (13). It is evident in the creatinine data from this study that glomerular filtration in the nephrectomized rats was still nearly 50% of that of sham-operated controls at 48 h. Previous studies have concluded that glomerular filtration was an important mechanism for leptin elimination, based on short-term studies of bilaterally nephrectomized rats and experiments in which the ureters of rats were occluded (6, 7). Our own studies modeling the kinetics of leptin suggested that leptin was primarily eliminated by the kidney by cellular uptake rather than by filtration (27). The dissociation of changes in glomerular filtration rate from leptin concentrations in nephrectomized animals followed over longer periods than in the earlier studies (6, 7) supports the view that cellular uptake, not glomerular filtration, is the predominant mechanism of renal leptin elimination. It is rapid adaptation of this uptake mechanism that may be responsible for the reestablishment of presurgical leptin concentrations in nephrectomized rats.

The observations of the present study cannot fully explain the results of studies of plasma leptin concentrations in ESRD patients (8, 11, 12, 18, 22, 26, 27). Although all studies have found elevated plasma leptin in ESRD patients, which is consistent with the important role of the kidney in leptin metabolism, a few studies have found that many ESRD patients had plasma leptin concentrations in the range of normals after their adiposity had been taken into account (18, 22). In one study, two subjects who were surgically anephric had normal leptin concentrations (18). Many ESRD patients are functionally anephric, with little or no residual blood flow to atrophied kidneys, but often have body mass index-appropriate leptin concentrations (18, 22). Therefore, adaptation of the elimination capacity of residual renal tissue, preliminarily observed in unilaterally nephrectomized rats, cannot account for the observations in ESRD patients, because these subjects have no residual renal tissue to make the adaptation. On the other hand, the highly effective adaptation observed in nephrectomized rats suggests that adaptive changes in elimination, rather than production, are likely the important mechanism that allows many ESRD patients to maintain body mass index-appropriate leptin concentrations.

Address for reprints: M. Landt, Dept. of Pediatrics, Washington Univ. School of Medicine, One Children’s Place, St. Louis, MO 63110.

Received 6 April 1998; accepted in final form 4 June 1998.

REFERENCES


