Mechanism of leptin removal from the circulation by the kidney

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Abstract

This study was performed to test the hypothesis that the kidneys play a primary role in the clearance of endogenous leptin from the circulation. Lean male Sprague-Dawley rats were anesthetized and subjected to various surgical manipulations of the kidneys. Sixty minutes after surgery arterial blood samples were taken at 1-h intervals for up to 8 h. Plasma leptin levels were determined by radioimmunoassay. Bilateral nephrectomy induced a rapid increase in plasma leptin concentrations above control values, indicating that the kidneys are important for the elimination of leptin from the circulation. Leptin was not metabolized across the renal circulation and was extracted intact by the kidney. Simultaneous measurement of renal plasma flow established renal leptin extraction at approximately 6.5 ng/min for both kidneys. Compared with the quantities extracted from the plasma, leptin was only present in the urine in small quantities, indicating extensive metabolic degradation in the renal tubules. High plasma leptin levels were not maintained after binephrectomy indicating that pathways other than the kidneys are also responsible for leptin clearance. Seven hours after bilateral ureteral ligation, a procedure which lowers glomerular filtration, plasma leptin levels were slightly elevated. The renal extraction of leptin did not change over a wide range of plasma leptin concentrations suggesting that renal leptin extraction is a high capacity, non-saturable process most probably glomerular filtration. Endogenous leptin is rapidly cleared from the circulation by glomerular filtration followed by metabolic degradation in the renal tubules.

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Introduction

Leptin, the cytokine product of the ob gene is secreted exclusively by white fat adipocytes (Zhang et al. 1994). Once released into the circulation, leptin lowers body weight by decreasing appetite and altering metabolic processes (Halaas et al. 1995, Pelleymouther et al. 1995, Rentsch et al. 1995, Cumin et al. 1996). Injection of leptin into the ventricular system of the brain reduces food intake and increases energy expenditure, presumably by enhancing sympathetic outflow (Campfield et al. 1995, Collins et al. 1996). The hypothalamus appears to be the major target tissue for the hormone, since leptin receptors are present within the appetite control centers of this region (Tartaglia et al. 1995, Mercer et al. 1996). While many of the actions of leptin are mediated by central mechanisms, the presence of leptin receptors in other tissues may also suggest that this peptide exerts effects on energy expenditure through peripheral sites (Tartaglia et al. 1995, Cioffi et al. 1996). Although the exact mechanism of action has not yet been elucidated leptin appears to be the long sought after satiety factor released in the periphery to regulate long-term body weight. In this lipostatic theory of body weight maintenance, as body fat increases more leptin is secreted, thereby bringing the fat mass back to a certain normal set point.

While it is well established that leptin enters the brain and binds to peripheral tissues, the metabolic fate of the large pool of circulating leptin remains largely unknown. Previous studies have shown that the kidneys play an important role in the elimination of small proteins. Indeed, other cytokines of similar size such as interleukin-2 are eliminated from the circulation primarily by the kidney (Maack et al. 1979, Gibbons et al. 1995). Recently published findings from our group have shown that high doses of exogenous leptin injected into the circulation are cleared mainly by the kidneys (Cumin et al. 1996). However, the mechanism by which endogenous leptin is eliminated from the circulation is largely unknown.

Therefore, the objective of the present study was to test the hypothesis that endogenous leptin is cleared from the circulation by the kidneys and to characterize the underlying mechanisms.

Materials and Methods

Animals

These studies were conducted primarily with lean male Sprague Dawley rats (Tif: RAlf [SPF], Novartis, Stein,
Switzerland) weighing 280–350 g. In some experiments Zucker fatty rats (fa/FA; Harlan-CPB, Austerlitz, Holland) weighing 450–550 g were also used. The rats were maintained in a room with a 12 h light/12 h darkness cycle (lights on from 0600–1800 h) at a temperature of 20–24 °C and monitored humidity. All animals were given tap water to drink and were maintained upon a normal pellet diet (NAFAG; Gossau, Switzerland) for at least 7 days before experiment to ensure standard levels of hydration.

**General surgical procedures**

Rats were anesthetized with Inactin (100 mg/kg; Byk Gulden, Konstanz, Germany) and placed into dorsal recumbency upon a heated surgical table maintained at 36 ± 0.5 °C. A tracheotomy was performed and the right femoral artery and vein were cannulated with polyethylene tubing (0.58 mm i.d. × 0.96 mm o.d.; Portex, Hythe, Kent, UK). The arterial and venous catheters were filled with heparinized (10 IU/ml; Liquemine, Roche-Pharma, Basel, Switzerland) isotonic saline. The arterial catheter was connected through an Isotec 6 PL pressure transducer (Health-Dyne, Marietta, GA, USA) to a Hellige Servomed pen recorder (Hellige, Freiburg, Germany) enabling a continuous record of the mean arterial pressure to be obtained throughout the experimental period. The femoral venous catheter was connected to a 20 ml syringe housed in an infusion pump (Precidor, Infors, Basel, Switzerland). The infusion pump delivered isotonic saline to the animal starting immediately after surgery and for the duration of the experimental period (1.2 ml/h). Blood samples were taken from the arterial catheter at different time points during the experiment and in each case were replaced with an equivalent volume of blood from a donor animal (details below). Evaporative losses in all experiments were minimized by placing saline-soaked cotton pads over the incision sites.

**Changes in plasma leptin after nephrectomy and ureteral ligation**

**Acute nephrectomy** Following the general surgical procedures described above, a midline abdominal incision was made to expose the viscera. A single 3–0 silk suture was tied tightly around each renal pedicle and the kidneys were removed by cutting through the renal vessels at a point distal to the ties. Following bilateral nephrectomy, the displaced abdominal contents were restored to their normal position and the abdominal incision closed. Sham control experiments were performed in the same way except that the sutures placed around the renal vessels were not tied.

**Acute ureteral ligation** Ureteral ligation was performed using the same basic surgical procedures described above. In these experiments, single 3–0 silk sutures were tied tightly around the ureters at a point 2 cm distal to the renal hilus. Sham control experiments were performed in the same way except that the sutures placed around the renal vessels were not tied.

**Experimental protocols** Thirty and sixty minutes after completing the surgical procedures for either bilateral nephrectomy or bilateral ureteral ligation arterial blood samples (400 µl) were taken into iced 1 ml syringes. Immediately after taking the 60 min blood sample, the kidneys were removed or the ureters ligated. Additional 400 µl arterial blood samples were taken at regular intervals over the following 4–8 h period. In some experiments involving bilateral nephrectomy, the intravenous saline infusion contained leptin which was delivered to the animals at either 0.5 µg/kg/min or 2.5 µg/kg/min for the duration of the experimental period. In these experiments control animals received the isotonic saline vehicle.

**Nephrectomy for 12, 24 and 48 hours** Rats were anesthetized with a mixture of 4% gaseous isofluran (Abbott AG, Cham, Switzerland) in 96% oxygen and both kidneys were removed or sham surgery performed as described above. Following surgery the animals were returned to their home cages and deprived of food. Either 12, 24 or 48 h after bilateral nephrectomy or sham surgery, the animals were anesthetized with isofluran and 6–8 ml blood was taken into an iced syringe through a 23 gauge needle inserted into the abdominal aorta. After the blood was taken the animals were killed with an intra-cardiac injection of Vetalarcol (Veterinaria AG, Zurich, Switzerland).

**Extraction of leptin across the kidneys**

**Surgical procedures** Rats were prepared as described above under General surgical procedures. In addition, either the left or right renal vein was exposed through a midline abdominal incision. The renal vein was punctured with a 1 cm long 26 gauge stainless steel tube and the tip advanced 0.5 cm into the vessel lumen. The catheter was held in place with tissue adhesive (Vetanarcol, Veterinaria AG, Zurich, Switzerland).

Following bilateral nephrectomy, the displaced abdominal contents were restored to their normal position and the abdominal incision closed. Sham control experiments were performed in the same way except that the sutures placed around the renal vessels were not tied.

**Percentage extraction of endogenous leptin across the left and right kidneys** At the end of the recovery period, blood samples (300 µl) were taken in random sequence from either the left or right renal vein and the arterial catheter.
Quantification of leptin extraction across both kidneys In these experiments the left ureter was cannulated and the femoral venous saline infusion contained $^3$H para aminohippuric acid (4-2 μCi/ml). Two hours after the end of the recovery period, urine was collected from the ureter into ice-cold microcentrifuge tubes over 2 consecutive 1-h periods. At the mid-point of each urine collection period, blood samples (400 μl) were taken from the arterial and renal venous catheters. The quantity of $^3$H present in the arterial, renal venous and urine samples was determined by liquid scintillation counting (Tri-Carb 2000 Liquid Scintillation Analyzer, Packard Instruments International SA, Zurich, Switzerland).

Percentage extraction of exogenous leptin across the left kidney These experiments were performed to study leptin extraction across the left kidney as described in the section Extraction of leptin across the kidneys. In addition, the intravenous saline infusion contained recombinant leptin which was infused at 5 or 50 μg/kg/min starting immediately after surgery and which continued for a 2-h period. At the end of the 2-h infusion period, blood samples (400 μl) were taken in random sequence from the aorta and left renal vein. Control experiments performed in lean or fatty Zucker rats received the isotonic saline vehicle.

Treatment of blood and urine samples The blood samples taken in all the above experiments were rapidly transferred to iced EDTA tubes, centrifuged at 4 °C and the plasma and urine stored frozen at −20 °C prior to assay of leptin or creatinine.

Leptin metabolism across the renal circulation Rats were prepared according to the methods described above under Extraction of leptin across both kidneys. The animals were infused with 0-5 μg/kg/min leptin dissolved in isotonic saline which was delivered via the femoral vein at a rate of 20 μl/min. The infusate also contained 5 μCi $^{125}$I-labeled leptin. The infusion continued for 10 min and then 400 μl blood samples were taken from both the femoral artery and the left renal vein. The blood samples were placed into iced EDTA tubes and centrifuged at 4 °C to obtain the plasma. In each arterial and venous sample, the iodinated leptin was separated from plasma proteins on 12% SDS-PAGE gels which were subsequently analyzed by phosphoimaging (Phosphoimager, Molecular Dynamics, Kemsing, Kent, UK). The stability of the iodinated leptin in each arterial and venous sample was assessed after densitometry analysis of the phosphoimage (Phosphoimager).

Blood donor animals Lean Sprague-Dawley rats were anesthetized with Inactin as described above and a midline abdominal incision performed to expose the abdominal aorta. Seven milliliters of blood were removed by direct aortic puncture and immediately placed into iced heparinized tubes. The blood was stored on ice and mixed prior to each injection.

Radioimmunoassay of plasma leptin Plasma leptin concentrations were determined by a previously described and characterized radioimmunoassay (Cumin et al. 1996). Polyclonal antibodies directed towards mouse leptin were raised in rabbits. Standards or unknown plasma samples (50 μl) were incubated together with $^{125}$I-leptin (50 μl), antisera (50 μl; dilution 1:4000) and 50 μl phosphate-buffered saline. The final mixture also contained 0-1% Tween 20 and 0-1% bovine serum albumin and was incubated at 4 °C for 18 h. The bound and free fractions were separated after adding 50 μl human γ-globulin (10 mg/ml in phosphate-buffered saline containing 0-1% Tween 20) and 500 μl 20% polyethylene glycol. The tubes were centrifuged for 30 min at 3000 g at 4 °C, after which the supernatant was aspirated and the pellet counted for $^{125}$I in a gamma counter (model 1277 Gamma Master, KLB Wallac, Turku, Finland). The detection limit of the assay was 0-48 ng/ml.

Measurement of plasma creatinine The concentration of creatinine in plasma was determined using a commercially available kit (Crea, F Hoffmann-La Roche, Basel, Switzerland).

Materials Recombinant mouse leptin was cloned and expressed by Novartis according to previously described methods (Rentsch et al. 1995). Leptin was iodinated using the lactoperoxidase method ([3-$^{125}$I-Tyr]-leptin, specific activity 68-1 TBq/mmol, ANAWA, Wengen, Switzerland) and had a purity of approximately 94%. p[Glycyl-2-$^{3}$H] aminohippuric acid (15–30 Ci/mmol) was also obtained from ANAWA.

Data analysis Renal plasma flow (RPF) was calculated as: $U_{PAH} \times V / A_{PAH} - RV_{PAH}$, where $U_{PAH}$ is the number of c.p.m. per ml urine, $V$ is the urine flow rate (ml/min), $A_{PAH}$ is the number of c.p.m. per ml arterial blood and $RV_{PAH}$ is the number of c.p.m. per ml renal venous blood.

Endogenous leptin clearance The renal extraction rate of leptin was calculated as RPF × $A_{leptin} - RV_{leptin}$. The fractional excretion of leptin (FE$^{leptin}$) was calculated as

$\text{FE}^{leptin} = \frac{\text{U}_{PAH} \times \text{V} - \text{U}_{PAH} \times \text{V}_{PAH}}{\text{V}_{PAH}}$
U_{\text{leptin}} \times$ the renal extraction rate and is expressed as percentage change, where $U_{\text{leptin}}$ is the quantity of leptin appearing in the collected urine per min.

**Statistical analysis**

Since many of the effects of the different treatment interventions were studied as a function of time, statistical analysis of the data was by analysis of variance for repeated measures. Where changes were not studied as a function of time statistical analysis was by analysis of variance or by paired $t$-tests. Post hoc analysis testing for differences between and within groups at different time points was conducted using the least significant differences procedure. $P<0.05$ was taken as the level of statistical significance in these studies.

**Results**

**Effect of acute bilateral nephrectomy on plasma leptin, plasma creatinine and blood pressure (Fig. 1)**

Plasma leptin levels measured 1 h after sham surgery averaged 1.20 ± 0.21 ng/ml and remained stable over the following 4-h period. Immediately following removal of both kidneys, leptin levels started to rise and had increased approximately 4-fold after 1 h. Four hours after bilateral nephrectomy, plasma leptin levels started to plateau at concentrations approximately 10 times higher than controls. Plasma creatinine levels measured 1 h after sham surgery averaged 58.4 ± 5.7 µmol/l and remained stable during the remainder of the experimental period. Immediately after bilateral nephrectomy plasma creatinine levels started to rise and were more than doubled 2 h later. Despite plateauing, plasma creatinine levels 4 h after removal of both kidneys were 2.5 times higher than measured in sham controls. Mean arterial pressure measured in the control and experimental groups of animals averaged 105.2 ± 3.93 and 111.4 ± 3.7 mmHg respectively. Blood pressure remained stable over the experimental period in the control group of animals and was not significantly affected by bilateral nephrectomy.

**Effect of bilateral nephrectomy for 12, 24 and 48 h on plasma leptin levels (Fig. 2)**

Plasma creatinine levels were similar in each of the groups of time control animals. Compared with control animals, bilateral nephrectomy for 12, 24 or 48 h produced a significant and progressive increase in plasma creatinine levels. Plasma leptin levels were similar in each of the groups of time control animals. Compared with controls, plasma leptin levels were significantly increased 12 h after bilateral nephrectomy. The increase in plasma leptin was less than observed 4 h after bilateral nephrectomy (Fig. 1).
indicating that the maximum increase in this parameter was reached at an intermediate time point. At increasing times after bilateral nephrectomy plasma leptin levels declined and were identical to control values in 48-h anephric rats.

**Effect of acute bilateral nephrectomy on plasma leptin levels during infusion of exogenous leptin (Fig. 3)**

Plasma leptin levels remained constant in each of the time control groups of animals infused either with vehicle or leptin (0.5 and 2.5 µg/kg/min). Except for magnitude, the pattern of the increase in plasma leptin levels was similar in each of the control and infusion groups following bilateral nephrectomy.

**Extraction of leptin from the circulation by the kidneys**

When compared with the arterial plasma concentration, the concentration of leptin was lower in the plasma of the left renal vein (Table 1). The percentage reduction in plasma leptin concentration measured across the renal circulation was similar for both the left and right kidneys (Fig. 4). Simultaneous measurement of the difference in leptin concentration across both kidneys and the renal plasma flow enabled the renal extraction rate to be calculated (6.5 ± 1.6 ng/min, Table 1). Of the leptin extracted by the kidneys only 3.1% appeared in the final urine.

**Leptin metabolism across the renal circulation**

The results in Fig. 5 show 12% SDS-PAGE gels of the radioactivity present in each of the arterial and venous blood samples. In each experiment, the total quantity of radiolabeled leptin present in the venous sample was always less than in the arterial sample. Densitometry readings of the arterial and venous blood samples indicated a renal extraction of 45 ± 3% (n=4). The 16 kDa band (16 kD), which corresponds to radiolabeled leptin constituted 100% of the total radioactivity present in each of the arterial and venous gels. Since there was no difference between the percentage of intact leptin present within the arterial and venous samples it can be concluded that the protein is extracted but not metabolized across the renal circulation.

**Effect of acute bilateral ureteral ligation on plasma leptin, plasma creatinine and blood pressure (Fig. 6)**

Plasma leptin levels were stable in the sham control experiments of this study. Bilateral ureteral ligation induced a significant increase in plasma leptin levels after 2 h which increased only slightly over the remainder of the experimental period. Plasma creatinine levels increased progressively above control levels starting 2 h after bilateral ureteral ligation. Bilateral ureteral ligation did not significantly affect the mean arterial pressure measured in these experiments.

**Percentage extraction of leptin across the left kidney at different plasma leptin concentrations (Fig. 7)**

The extraction of leptin across the left kidney in Zucker fatty rats, normal lean rats and in normal lean rats infused intravenously with 5 and 50 µg/kg/min leptin are shown. The results show that the renal extraction of leptin remained constant at approximately 25% across a wide range of plasma concentrations.

**Discussion**

The purpose of these studies was to test the hypothesis that endogenous leptin is removed from the circulation primarily by the kidneys.

Creatinine is formed as a by-product of muscle metabolism. After secretion into the circulation, creatinine is removed from the plasma by the kidneys primarily by
glomerular filtration (Mitch et al. 1980, Bauer et al. 1982). Under normal conditions the rate of excretion is equal to the rate of formation and is the basis of the stable baseline plasma creatinine levels observed in these studies. Since creatinine is removed from the blood primarily by the kidneys, bilateral nephrectomy produced the expected rise in plasma creatinine levels.

Immediately following bilateral nephrectomy, plasma leptin levels increased, suggesting that leptin is eliminated primarily by the kidneys. Plasma leptin levels started to plateau 3 h after bilateral nephrectomy and had returned to control levels after 48 h. An explanation for the ultimate reversal of the high plasma leptin levels observed following bilateral nephrectomy may be directly related to the increase in endogenous plasma leptin levels which may act to inhibit further protein release from the adipocytes. Alternatively, elimination pathways other than the kidneys may become progressively of greater importance over time in binephrectomized animals. Some light may be shed on these explanations by the experiments in which exogenous leptin was infused into anephric rats. In these studies, constant intravenous infusion of leptin at either 0.5 or 2.5 µg/kg/min resulted in an increase in plasma leptin levels similar, except in magnitude, to those of the endogenous peptide observed after binephrectomy. Consequently, the plateauing of leptin levels in anephric rats cannot be due to inhibition of leptin release from the adipocytes but rather to the progressive importance of other elimination mechanisms of which the liver is the most likely candidate (Lau et al. 1996).

Leptin circulates in both bound and free forms within the circulation. In theory, removal of the kidneys or bilateral ureteral ligation could affect the plasma levels of leptin binding proteins. Since it has not been established whether the free or bound form of leptin is

![Figure 3 Effect of acute bilateral nephrectomy on plasma leptin levels during infusion of exogenous leptin. Rats underwent bilateral nephrectomy (□) or sham surgery (■) and were infused with differing doses of recombinant leptin or vehicle. Blood samples were taken at various time points and analyzed for leptin. Results are expressed as means ± s.e. In every case the symbols are larger than the s.e. bars. Numbers of experiments are in parentheses. Statistics by repeated measures analysis of variance; post hoc test by the least significant difference procedure which compared changes between groups at each time point. • P<0.05, significantly different from time 0; ◇ P<0.05, significant difference between groups at each time point.](image)

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<th>2</th>
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![Table 1 Renal handling of leptin. Results are expressed as means ± s.e. (n=5)](table)

<table>
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<tr>
<th>A_leptin (ng/ml)</th>
<th>V_leptin (ng/ml)</th>
<th>A–V (ng/ml)</th>
<th>A–V (%)</th>
<th>RPF (ml/min)</th>
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<th>A–V (ng/ml)</th>
<th>U_leptin (ng/µl)</th>
<th>Uvol (µl/min)</th>
<th>U_leptinV (ng/min)</th>
<th>Uvol (µl/min)</th>
<th>FE_leptin (%)</th>
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<td>1.8 ± 0.4</td>
<td>−30.5 ± 4.3</td>
<td>0.7 ± 0.1</td>
<td>8.8 ± 0.9</td>
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<td>0.018 ± 0.004</td>
<td>10.6 ± 1.0</td>
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<td>3.1 ± 0.8</td>
<td>10.6 ± 1.0</td>
<td>0.178 ± 0.038</td>
<td>3.1 ± 0.8</td>
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A_leptin = arterial leptin concentration; V_leptin = venous leptin concentration; A–V = arterial–venous difference; RPF = renal plasma flow; Renal extraction = A_leptin – V_leptin × RPF; U_leptin = urine leptin concentration; Uvol = urine volume; U_leptinV = urine leptin excretion; FE_leptin = fractional leptin excretion.
recognized by the antibodies. Changes in circulating binding proteins could, in part, account for the changes in apparent plasma leptin concentration observed in these experiments.

In these studies both the left and the right kidney extracted approximately 25% of the leptin entering from the renal arteries. Since iodinated leptin was not metabolized across the kidney, extraction of the complete protein must occur on passage through the renal circulation. Based upon the prevailing arterial plasma concentration in these animals approximately 390 ng leptin are removed from the plasma by both kidneys per hour. Since stable plasma levels were observed in these experiments it follows that the adipocytes must continuously secrete equivalent quantities of leptin into the circulation.

Compared with the amount extracted by the kidney very little intact leptin was found in the final urine. The calculated fractional excretion of leptin suggests that 97% of the extracted leptin is metabolized by the kidney. After removal from the circulation by the kidneys all small proteins such as leptin are extensively metabolized and the constituent amino acids reabsorbed into the bloodstream. This mechanism serves to ensure that filtered proteins and amino acids are not continually lost into the urine.

The elimination of leptin from the circulation by the kidneys could be mediated by glomerular filtration and/or tubular secretion: two distinct but ultimately additive pathways. The glomerular membrane completely restricts the passage of molecules with a molecular mass greater than 50 kDa while increasingly favoring the passage of proteins of decreasing size (Skorecki et al. 1986). Since molecules with a molecular mass of 15–17 kDa are hindered only slightly in their passage across the capillary wall it follows that leptin with a molecular size of 16 kDa would be essentially freely filtered (Skorecki et al. 1986). This conclusion must be tempered, however, by the fact that an acidic protein such as leptin bearing negative charges would be expected to show some restriction in its passage across the glomerular membrane (Chang et al. 1975, Zhang et al. 1994).

Theoretically, the difference between the measured filtration fraction and the percentage extraction of leptin could indicate the proportion of the clearance that is mediated by glomerular filtration. The renal filtration fraction has been measured in rats and shown to be approximately 30%, a value larger than the renal extraction of leptin (Alavi et al. 1995). It is possible, therefore, that glomerular filtration could fully account for the removal of leptin from the circulation in these studies.

To determine whether glomerular filtration plays the predicted role in the renal elimination of leptin, initial experiments were carried out in animals subjected to bilateral ureteral ligation. Bilateral ureteral ligation reduces

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**Figure 4** Extraction of leptin from the circulation by the kidneys. Blood was taken from the aorta as well as the left and right renal veins and assayed for leptin. Results are expressed as means ± s.e. Left kidney (open bar) (n=4), right kidney (hatched bar) (n=5). Baseline plasma levels were 1.20 ± 0.13 ng/ml (n=9). Statistics by paired t-tests which compare arterial plasma leptin concentrations with those in either the right or left renal veins. *P<0.05, significant difference between leptin concentrations in artery and vein.

**Figure 5** Leptin metabolism across the renal circulation. 12% SDS-PAGE gels of the radioactive leptin present in the arterial and renal venous blood samples. A=artery; V=vein.
glomerular filtration by increasing tubular pressure (Dal Canton et al. 1977). Following bilateral ureteral ligation in these experiments, plasma leptin levels increased by 30–40%, a value much less than the 700% increase observed after bilateral nephrectomy. There are several possible explanations for the finding that ureteral ligation did not affect plasma leptin levels to the same extent as nephrectomy. First, renal mechanisms may exist that operate independently of glomerular filtration. The active secretion of leptin from the blood to the tubular fluid would be an example. In this regard leptin receptors have been shown to exist in the kidney and in theory could function there as transport molecules (Tartaglia et al. 1995). A second possibility is that glomerular filtration, although reduced to very low levels, is not completely blocked by ureteral ligation. In this regard it has been well characterized that even several hours after bilateral ureteral ligation enough tubular fluid reabsorption occurs to drive a small quantity of glomerular filtration (Harris & Gill 1981, Klahr et al. 1986). Under these conditions leptin could make its way into the renal tubules in quantities sufficient to influence the rate of systemic elimination. Thus,

Figure 6  Effect of acute bilateral ureteral ligation on plasma leptin, plasma creatinine and blood pressure. Following baseline measurements, rats underwent bilateral ureteral ligation (■) or sham surgery (■) and the changes in each measured parameter followed as a fraction of time. Results are expressed as means ± S.E. from five experiments in each control and experimental group. In some cases the symbols are larger than the S.E. bars. Statistics by repeated measures analysis of variance; post hoc test by the least significant difference procedure which compared changes from baseline and changes between groups at each time point. ○ P<0.05, significantly different from time 0; ◆ P<0.05, significant difference between groups at each time point.

Figure 7  Percentage leptin extraction across the left kidney at different plasma leptin concentrations. The percentage extraction of leptin was measured across the left kidney of Zucker rats, normal rats and in normal rats infused with either 5 or 50 µg/kg/min leptin. Results are expressed as means ± S.E. Numbers in parentheses are the number of animals. Statistics by analysis of variance did not detect a significant difference in the percentage extraction of leptin between the four groups.
while studies in ureterally ligated animals can suggest the involvement of glomerular filtration in the elimination of leptin they cannot exactly define the extent of this process.

To define the role of glomerular filtration further, renal leptin extraction was measured at increasing plasma leptin levels. If leptin is extracted from the renal circulation by active secretion into the renal tubules then it should be possible to demonstrate saturation of this transport mechanism at high plasma leptin concentrations. Saturation of active leptin secretion within the kidney would appear as an apparent decrease in the percentage renal extraction of leptin. However, the percentage renal extraction of leptin was unchanged even at plasma levels of leptin several orders of magnitude greater than normal circulating levels. These observations strongly suggest that renal leptin extraction is a high capacity non-saturable process consistent with glomerular filtration.

Recently, leptin was shown to be elevated in the plasma of patients with end-stage renal disease and chronic renal failure (Iida et al. 1996, Merabet et al. 1997, Sharma et al. 1997). These results suggest that the kidneys also play an important role in the elimination of leptin in man.

In conclusion, the kidneys play an important role in the systemic elimination of circulating leptin. Leptin is not metabolized across the kidney and is therefore extracted as an intact protein from the renal circulation. The mechanism involved is a high capacity non-saturable process, most probably glomerular filtration.

References


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