Variation in Plasma Leptin Concentrations After Unilateral Nephrectomy

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The temporal changes in plasma leptin concentrations were studied in healthy adults who underwent unilateral nephrectomy. Another group who underwent abdominal surgery for repair of aneurysm or to relieve arterial stenosis, was also studied. Plasma leptin concentrations increased to 230% ± 74% of prenephrectomy levels at 8 to 16 hours after surgery and then generally declined. Subjects with prenephrectomy leptin concentrations above 14 μg/L maintained elevated postnephrectomy levels, whereas subjects with low prenephrectomy concentrations had final leptin levels below prenephrectomy concentrations. Abdominal surgery subjects did not manifest the increase after surgery, but generally had declining concentrations throughout the convalescent period. Free and bound fractions of plasma leptin and leptin binding capacity were measured in the prenephrectomy and peak specimens (8 to 16 hours postnephrectomy) by high-performance liquid chromatography (HPLC). The increase in total leptin postnephrectomy largely affected the free fraction of leptin, without significant increase in bound leptin or leptin binding capacity. We conclude that (1) plasma leptin concentrations increase acutely after nephrectomy, consistent with the role of the kidneys in eliminating circulating leptin; (2) plasma leptin concentrations decline thereafter, suggesting activation of compensatory elimination capacity; and (3) the postnephrectomy peak in total leptin increases primarily free leptin.

LEPTIN, A RECENTLY discovered hormone produced by adipose tissue, circulates in the blood and interacts with hypothalamic centers which control food intake (for review, see Friedman and Halaas[1]). Many studies have shown that the hormone is primarily eliminated from the circulation by the kidneys.[2-11] Arteriovenous balance studies found that 12% to 26% of plasma leptin is removed by a single pass through the kidneys.[2-4] Kinetic studies in rats given radioactively labeled leptin produced models, which suggested that the kidneys accounted for more than 80% of the elimination of leptin.[5-6] Several cross-sectional studies of leptin concentrations in patients with impaired kidney function demonstrated elevated levels.[2,7-11]

A previous study of leptin concentrations in unilaterally nephrectomized rats found that leptin increased immediately after nephrectomy to reach peak concentrations about 6 to 8 hours following surgery, but then rapidly returned to prenephrectomy levels.[12] Creatinine concentrations remained elevated long after leptin had returned to baseline, suggesting that it was not restoration of overall renal function, which accounted for the return to baseline. Kinetic studies of the disappearance of ^{125}I-labeled leptin from circulation showed that production of leptin was unaltered by unilateral nephrectomy, suggesting that a compensatory, elimination of leptin was responsible for the rapid restoration of prenephrectomy levels.[12]

Leptin is known to circulate in both protein-bound and free forms; there are several binding proteins, including a truncated form of the leptin receptor.[13] Comparatively little is known about how free and bound forms of leptin are effected by circumstances, which acutely alter total leptin concentrations, such as nephrectomy. These findings prompted us to study total, as well as free and bound leptin concentrations, in human volunteer kidney donors, who were generally healthy, but had a much greater variation in body composition than the rats used in the previous study. The results suggest that in humans, as well as rodents, unilateral nephrectomy is accompanied by the rapid appearance of compensatory elimination mechanisms.

MATERIALS AND METHODS

Patients

Volunteer participants (n = 14; 6 males) were recruited from 2 groups: (1) the "nephrectomy" group (n = 8; 4 males) was comprised of healthy donors of kidneys to related recipients, and (2) the "abdominal surgery" group (n = 6; 2 males) were ambulatory patients undergoing vascular surgery of the arterial system. The abdominal surgery group was older, but the 2 groups were otherwise similar in several pertinent characteristics (Table 1). No participant had evidence of renal disease, as evidenced by a serum creatinine above 115 μmol/L.

In the nephrectomy group, surgery was performed using a standard open technique via a flank incision. The donors received 3 to 6 L of normal saline intraoperatively, as well as 12.5 to 25 g of mannitol, 2,500 to 5,000 U of heparin, and 10 to 20 mg of furosemide just prior to removal of the kidney. General anesthesia was augmented by epidural anesthesia as chosen by the patient and anesthesiologist. Postoperative narcotic analgesia was provided, either by epidural infusion, patient-controlled analgesia devices, oral narcotics, or a combination of these techniques. Recovery was uneventful, and subjects were discharged 3 to 5 days after surgery. Patients received glucose infusion postoperatively, until normal oral feeding resumed, usually on the second postoperative day.

The abdominal surgery patients underwent aortic revascularization for either aneurysmal or occlusive disease. In patients with aneurysmal disease, both common iliac arteries were mobilized, as well as the infrarenal aortic neck. Patients undergoing aneurysm repair had the aortic replacement with a tube Dacron graft (Impra Corp, Tempe, AZ),

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Table 1. Comparison of Characteristics for the Nephrectomy and Vascular Surgery Groups

Characteristic	Nephrectomy (n = 8)		Vascular Surgery (n = 6)		
	$\bar{x} \pm$ SD	(range)	$\bar{x} \pm$ SD	(range)	*P**
Age (yr)	30.5 ± 6.6	(19-39)	58.5 ± 13.7	(36-72)	< .001
BMI (kg/m^2)	23.2 ± 4.0	(16.5-27.8)	25.6 ± 4.1	(22.6-33.4)	.31
Initial leptin (μg/L)	12.5 ± 9.6	(1.0-29.7)	19.9 ± 9.2	(8.3-33.2)	.18
Initial creatinine (μmol/L)	80 ± 18	(71-115)	97 ± 18	(53-115)	.23

* Statistical significance was assessed with Student's 2-tailed *t* test. All comparison data were normally distributed.

while patients with aortoiliac occlusive disease underwent aortobifemoral bypass grafting using bifurcated Dacron grafts. During surgery, patients received Lactated Ringer's solution. Postoperatively, patients received 5% glucose in Lactated Ringer's solution, which generally occurred at 150 cc/h until the patient tolerated oral feeds, which generally occurred by the third postoperative day. All patients recovered in the surgical intensive care unit for approximately 24 hours, and hospital length of stay was 5 to 7 days.

All participants gave informed consent, in accordance with a protocol approved by the Human Studies Committee of Washington University.

Study Protocol

Blood specimens needed for the study were collected from either a catheter placed in the hand vein for this purpose (nephrectomy group) or from a central line placed at the time of surgery (abdominal surgery group). Specimens collected 1 to 7 days before surgery (average, 1.9 days) for purposes of blood typing served to establish presurgical leptin concentrations. Postsurgically, blood specimens (3 mL) with EDTA anticoagulant were collected at 4, 8, 12, 24, 36, 48, and 72 hours; in a few instances in the nephrectomy group, occlusion of the catheter by clotting resulted in incomplete data sets. Height and weight determined at the time of admission were used for calculation of body mass body mass index (BMI).

Analyses

Leptin was determined in plasma with a commercial radioimmunoassay (Linco Research, St Charles, MO) as previously described.[14] Creatinine was measured in the same plasma specimens by a standard clinical laboratory analyser, the Vitros 250 (Ortho-Clinical Diagnostics). The protein-bound and free fractions of plasma leptin were measured by high-performance liquid chromatography (HPLC) chromatographic separation as previously described.[15]

Statistical Analysis

All results are presented as means ± 1 SD, unless otherwise stated. Statistically significant differences were assessed with a 2-tailed Student's *t* test for unpaired data; when the data were not normally distributed, then the Mann-Whitney rank sum test was applied. For paired data, a repeated measures 1-way analysis of variance (ANOVA) test was performed. When the paired data were not normally distributed, then the Friedman repeated measures ANOVA rank test was used. A comparison was regarded as significantly different when *P* values were less than .05.

RESULTS

The pattern of changes in leptin concentration in plasma was followed over the 72 hours following unilateral nephrectomy (Fig 1A). In each of the nephrectomy subjects, plasma leptin peaked at 8 to 16 hours postnephrectomy, and thereafter declined, although the extent of decline varied widely. The mean plasma leptin presurgically was 12.5 ± 9.6 μg/L, which increased to 29.5 ± 25.9 μg/L at 8 to 16 hours ($P = .028$). When the peak concentrations are converted to percent of presurgical concentrations, then the peak concentrations were 230% ± 74% of presurgical levels ($P = .002$). The leptin concentrations in the last specimen (collected 41 to 70 hours postnephrectomy) averaged 17.1 ± 19.1 μg/L ($P = .272$), which was 111% ± 54% of the starting concentration ($P = 1.00$). The peak increase did not appear to depend on the initial leptin concentra-

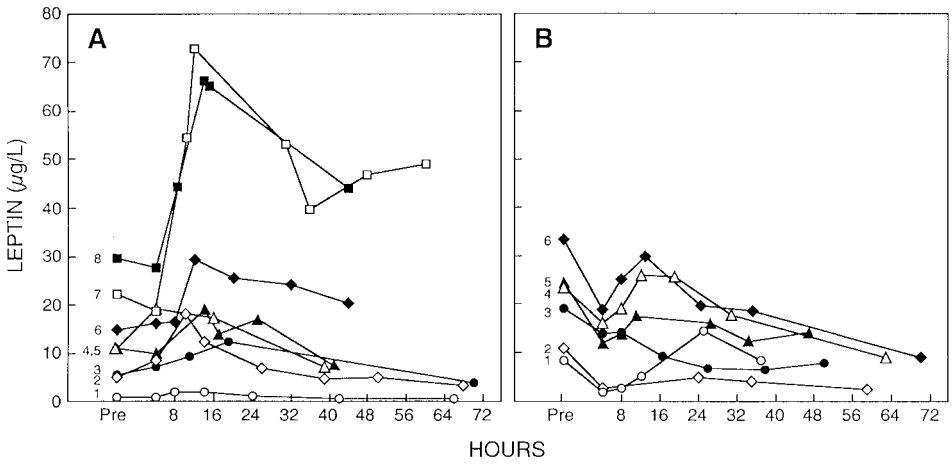

Fig 1. (A) Pattern of changes in plasma leptin concentration after unilateral nephrectomy. Eight subjects provided serial blood specimens just before (Pre) and for up to 72 hours following surgery. (B) Pattern of changes in plasma leptin concentrations after abdominal surgery (aneurysm repair or relief of arterial stenosis), but without impairment of renal function. Six subjects provided serial blood specimens before (Pre) and for up to 72 hours following surgery.

tion; the peak levels for 3 nephrectomy subjects with the lowest presurgical leptin concentrations ($\bar{x}$ = 3.8 μg/L) were 248% of the presurgical levels compared with 249% for the 3 subjects with the highest presurgical concentrations ($\bar{x}$ = 22.3 μg/L). A plot of the time courses of leptin concentration for the 8 nephrectomy subjects provided 2 interesting observations (Fig 1A). First, leptin levels were largely unchanged at 4 hours postnephrectomy, and in 3 of the 8 subjects, the 4-hour level was slightly lower than the presurgical level. Second, the decline of leptin after peaking 8 to 16 hours postnephrectomy was different for those with high prenephrectomy leptin concentrations (>14 μg/L) compared with subjects with lower presurgical concentrations. Females and subjects with larger BMI generally had the higher prenephrectomy leptin concentrations. Whereas subjects with prenephrectomy leptin concentrations below 14 μg/L (subjects 1 to 5 of Fig 1A) had declines (after peaking at 8 to 16 hours) that brought their final concentrations below their presurgical levels, the 3 subjects (subjects 6 to 8 of Fig 1A) with presurgical concentrations above 14 μg/L had final concentrations that were 168% of their presurgical levels. Plasma creatinine increased from 80 ± 18 μmol/L presurgically to reach peak levels at 8 to 16 hours (115 ± 18 μmol/L) but, in contrast to the temporal pattern of leptin, remained elevated in the final specimens collected 41 to 72 hours postsurgically (115 ± 18 μmol/L).

The pattern of changes in plasma leptin concentrations in abdominal surgery subjects, who underwent similar surgery, but did not have any change or compromise in renal function, was different than the nephrectomy group. Presurgical leptin concentrations were 19.9 ± 9.2 μg/L, and decreased to 16.2 ± 10.4 μg/L at 8 to 16 hours postsurgery (P = .045), in contrast to the nephrectomy subjects in which an increase was observed. Final leptin concentrations (37 to 68 hours postsurgery) decreased even further (8.4 ± 3.7 μg/L) relative to presurgical levels (P = .017). A plot of leptin concentration versus postsurgical time (Fig 1B) showed a very different pattern compared with the nephrectomy subjects. In all subjects, leptin concentrations decreased at 4 hours postsurgery, as it had in some of the nephrectomy subjects, but the decreases were of greater magnitude. In most abdominal surgery subjects, leptin concentrations at 8 to 16 hours postsurgery were higher than the levels at 4 hours, but the peak levels were increased modestly (155% of the 4-hour concentrations). Leptin concentrations generally trended lower throughout the remainder of the recovery period. There was no discernable difference in the pattern between abdominal surgery subjects with presurgical leptin greater than 14 μg/L and those with lower concentrations. Plasma creatinine concentrations decreased from a presurgical level of 98 ± 18 μmol/L to 80 ± 18 μmol/L 8 to 16 hours after surgery and to 71 ± 18 μmol/L in the final specimens. In each of the 6 subjects, creatinine was reduced from presurgical concentrations, in contrast to the nephrectomy subjects in which creatinine increased in each instance.

Plasma from the nephrectomy subjects was subjected to HPLC analysis of free and bound fractions of leptin and measurement of leptin binding capacity (a measure of leptin binding protein concentrations). Specimens collected presurgically and at the peak in postnephrectomy increase (8 to 16 hours postnephrectomy) were analyzed to determine if the increase in total leptin caused by nephrectomy effected primarily the free or bound fractions, or both. Both bound and free leptin were increased in the 8- to 16-hour postsurgery specimens, but the increase in free leptin was much larger (Table 2), and only the increase in free leptin was significant (P = .042). Potential increase in bound leptin was restrained by the limited amount of unused binding capacity present (binding capacity was 76% saturated in the presurgical specimens) and the fact that binding capacity was similar in the presurgical and peak specimens (Table 2). Thus, the saturation of the binding capacity in the peak specimens was nearly complete (93%).

Table 2. Free Leptin, Bound Leptin, and Leptin Binding Capacity in Specimens Collected Before, and 8 to 16 Hours After Unilateral Nephrectomy (n = 8)

	Presurgery	8 to 16 Hours Postsurgery	P
Free (μg/L)	8.7 ± 8.0	28.2 ± 30.8	.042
Bound (μg/L)	1.6 ± 1.1	2.3 ± 1.1	.108
Binding capacity (μg/L)	2.1 ± 0.8	2.4 ± 1.3	.47

DISCUSSION

These results demonstrate that in humans, as in previous experiments in rats,[12] unilateral nephrectomy is accompanied by an acute increase in plasma leptin concentrations, consistent with the hypothesis that the kidneys are the primary means of eliminating leptin from the circulation.[2-11] Removal of one half of kidney function roughly doubled (at the peak concentration after surgery) the leptin concentration, presumably because leptin production remained constant, and elimination capacity was reduced by half. The ratio of peak to presurgical leptin concentrations was insensitive to the initial leptin level, averaging more than 240% in subsets of subjects with the highest and lowest prenephrectomy concentrations. Leptin concentrations reached peak levels 8 to 16 hours postnephrectomy, which is delayed in comparison to results in rats, in which peak levels were reached at 6 to 8 hours[12]; however, the half-life of leptin in human plasma of about 25 minutes[16] is more than twice that of rats (9 minutes).[12] Thus, the slower kinetics of leptin in humans could account for the greater time period needed to reach peak levels after nephrectomy.

Leptin concentrations decreased rapidly after reaching a peak in most subjects, with presurgical levels restored by 36 hours in most subjects. This rapid return to baseline concentrations likely reflects activation of compensatory elimination capacity, since kinetic studies in rats showed that production of leptin was not decreased in response to unilateral nephrectomy.[12] The time course of leptin concentration changes in humans was variable, with prolonged elevations seen in subjects with higher presurgical concentrations. The reason for the disparity in time course is unknown, but one possibility is that the compensatory elimination that arises following nephrectomy is of such limited capacity that it is unable to fully compensate when initial concentrations (combined with the increase following nephrectomy) are relatively high. Further study of more subjects, selected for a range of body composition and incorporating kinetic studies, are needed to establish this hypothesis.

The time course of changes in leptin concentrations following abdominal surgery (without nephrectomy) was dissimilar to the pattern observed in nephrectomy subjects. Concentrations generally trended lower throughout the postsurgical period, with consistent decreases in the first 4 hours postsurgery. There was a small and inconsistent peak of leptin level at 8 to 16 hours postsurgery compared with the 4-hour concentrations. The relevance of this observation to the larger peak observed following nephrectomy is unclear, but it did not appear to be related to decreased renal function, because creatinine levels were equal or lower in each instance compared with either presurgical or 4-hour postsurgical levels. The abdominal surgery procedures were generally more traumatizing than nephrectomy, as evidenced by the longer postoperative stay and longer period in intensive care. It is possible that the stress or trauma of abdominal surgery promotes a modest increase in leptin postsurgically, and therefore makes some contribution to the large postsurgical elevation observed in the nephrectomy subjects. Similar modest increases were observed in 2 other studies of traumatic surgery.[17,18] In the study of nephrectomized rats,[12] postsurgical elevations were not observed in sham-operated controls. Abdominal surgery subjects also received extended fluid therapy compared with the nephrectomy subjects, and their specimens were drawn from a central line instead of a catheterized hand vein. It is not clear that differences in fluid therapy between the groups had any role in the observed differences in postsurgical leptin, since the fluid therapy of the 2 groups was similar in the 24 hours postsurgery, when the differences in leptin were most pronounced. Leptin concentrations in specimens collected from a hand vein are likely very similar to venous specimens collected from a central line, and any small differences would not affect the trend in postsurgical changes in leptin concentrations in individual subjects.

Chromatography to determine free and bound leptin fractions of plasma leptin showed that nearly all of the postnephrectomy increase in leptin was free leptin. Previous studies have established that obese individuals have most of their leptin in the free form, and lean individuals have a much higher fraction of their total plasma leptin in the bound form.[13,15] There has been only a single study that examined changes in free/bound leptin under conditions that acutely alter leptin, which examined the changes during a 24-hour fast.[13] That study found that the decrease in total leptin, which accompanies fasting, significantly effected only the free fraction, although a large (but statistically insignificant) decrease in bound leptin was also observed. We have recently examined the effect of a 22-hour fast on free/bound leptin concentrations and found significant decreases in both free and bound leptin (M Landt, personal communication, April 2001). The dynamics of bound leptin and whether it changes acutely in concert or independently of the free fraction of plasma leptin is not understood, but the evidence from our nephrectomy studies suggests that bound leptin does not change acutely under conditions in which free leptin concentrations are increasing rapidly.

One important constraint on acute increases in bound leptin is the availability of unoccupied binding capacity (excess binding proteins). Because most of the leptin binding capacity of our nephrectomy subjects was already bound to leptin in the prenephrectomy state, large increases in bound leptin that matched the doubling in total leptin occurring postnephrectomy would require the generation of additional leptin binding capacity (leptin binding proteins). Leptin binding capacity was unchanged postnephrectomy, suggesting that the processes, which synthesize the binding proteins, are relatively insensitive to acute changes in leptin concentration, at least for the extent and period of increase that accompanies unilateral nephrectomy.

The physiologic effect of acutely doubling plasma leptin following nephrectomy, which might be expected to alter appetite or metabolic rate, appeared to be absent. Although we did not try to quantify measures of appetite such as food intake, we did not discern any subjective changes that correlated with the pattern of changes in leptin postsurgically. Current theory, which takes into account the "resistance" of obese subjects to the very high levels of circulating leptin[19,20] and the ability of exogenous leptin to blunt the neuroendocrine effects of fasting,[21] suggests that leptin acts physiologically primarily to oppose acute or chronic loss of adipose stores during periods of low food intake.[22] This theory holds that the acute decrease in plasma leptin, which accompanies fasting, initiates profound changes in appetite, as well as in the levels of adrenocortical and reproductive hormones, but that the response to increases in leptin, such as those accompanying increased adipose stores, are muted to favor the accumulation of adipose tissue in times of plentitude. Our subjective assessment in our postnephrectomy subjects is in accord with this theory.

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