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Pharmacokinetics of human leptin in mice and rhesus monkeys

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OBJECTIVE: The pharmacokinetic characteristics of human leptin were examined in rhesus monkeys and in C57BL/6J mice fed a normal chow or a high-fat diet.
DESIGN: For the *monkey study*, in nine rhesus monkeys (body weight 12.4 ± 2.4 kg; mean ± s.d.), recombinant met-human leptin was injected intravenously or subcutaneously (1 mg/kg). For the *mouse study*, after 6 months of feeding C57BL/6J mice a high-fat diet (body weight 32.9 ± 3.6 g; $n = 8$) or a control diet (24.5 ± 1.2 g; $n = 6$), recombinant met-human leptin was administered intraperitoneally (10 μg/g). Blood samples were collected for leptin measurement at specific time points after leptin administration.
MEASUREMENTS: Plasma leptin concentrations were determined by radioimmunoassay and pharmacokinetic analysis was performed.
RESULTS: Disposition of human leptin in rhesus monkeys was biphasic following intravenous administration, with a terminal phase half-life of 96.4 ± 16.5 min and clearance of 1.8 ± 0.2 ml/min/kg. Subcutaneously administered leptin was absorbed slowly, perhaps by a zero-order process as leptin levels appeared to plateau and remained elevated throughout the 8 h sampling period. In C57BL/6J mice, the absorption and elimination of human leptin were both first-order following intraperitoneal administration. Pharmacokinetic parameters did not differ between normal-weight mice fed a chow diet and obese mice fed a high-fat diet. The elimination half-life was 47.0 ± 26.4 min in mice fed a high-fat diet and 49.5 ± 12.0 min in mice fed a control diet.
CONCLUSION: The kinetics of leptin in rhesus monkeys were biphasic and clearance was similar to values previously reported in humans. The estimated half-life was 96.4 min in rhesus monkeys and 49.5 min in normal weight mice. The was no difference in leptin kinetics between high-fat fed and control mice, suggesting that the increased baseline leptin levels in the obese mice are due to increased leptin production and secretion.
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Keywords: leptin; pharmacokinetic; monkeys; mice

Introduction

Leptin is encoded by the *ob* gene and expressed mainly in adipose tissue.[1,2] It is released from the adipocytes in proportion to recent energy intake[2–5] and acts primarily in the central nervous system to reduce food intake and stimulate energy expenditure, thereby participating in the regulation of energy balance and body weight.[6–8] In obesity, the circulating levels of leptin are increased, which has been thought to be due to increased synthesis and release from the adipocytes as well as increased adipose mass *per se*.[2,9–11] Leptin production is regulated by insulin,[12,13] an effect that appears to involve insulin's action to increase glucose metabolism in adipose tissue.[14] In humans, circulating leptin concentrations are correlated with circulating insulin[15,16] and 24 h circulating leptin levels are increased after high-carbohydrate meals, which induce larger postprandial insulin and glycemia excursions than high-fat meals.[17] However, the extent to which changes of circulating leptin kinetics contribute to variations of leptin observed with changes of adiposity, energy intake, insulin or dietary macronutrient composition is not clear, in part because the pharmacokinetics of circulating leptin are not well characterized.

Previous studies using radiolabeled leptin have reported significantly different leptin plasma half-lives, both across and within species. A study in humans reported a half-life of circulating leptin of 25 min.[18] Two studies in mice suggested the considerably longer half-lives of 1.5 and 3 h,[19,20] whereas a recently published study in Zucker rats reported a much shorter half-life of circulating leptin (~6 min) with no difference between lean and obese animals.[21] Much of the disparity in the literature is attributable to a wide range of methodologies and study objectives, with half-life being determined secondarily, often based on a minimal number of time points. In this study, we have examined the pharmacokinetic characteristics of human leptin after intravenous or subcutaneous administration to rhesus monkeys. To our knowledge, the pharmacokinetics of leptin have not been previously characterized in nonhuman primates. As it has been suggested that alterations in leptin kinetics may account for the elevated plasma leptin

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levels observed with obesity, we have also compared the pharmacokinetic characteristics of recombinant human leptin after a single intraperitoneal injection in C57BL/6J mice fed either a normal chow diet or a high-fat diet. Mice with increased adiposity resulting from high-fat feeding were examined because it is known that circulating levels of leptin are markedly elevated by the high-fat feeding in conjunction with the development of moderate obesity and severe glucose intolerance.[22–25] Thus, this model allowed us to observe potential changes in leptin pharmacokinetics occurring in these animals with markedly elevated steady-state levels of endogenous leptin. Pharmacokinetic parameters were established by compartmental and noncompartmental analysis of the circulating leptin levels after a single intraperitoneal, intravenous or subcutaneous injection.

Methods

Monkey study

Nine healthy adult (age 7–17 y) male rhesus monkeys (*Macaca mulatta*) weighing between 8.9 and 16.0 kg (12.4 ± 2.4 kg; mean ± s.d.) were used for these studies. Prior to selection for the study, a physical examination, complete blood count and serum biochemistry panel were performed on each animal. The animals had previously been acclimated to several hours of chair restraint with a minimum of 10 training sessions.[26] Animals were housed in the AAALAC accredited facilities of the California Regional Primate Research Center (CRPRC) in accordance with standards established by the US Animal Welfare Act and the Institute of Laboratory Animal Resources (ILAR). The experimental protocols were approved by the institutional animal care and use committee at the University of California Davis and the CRPRC. Studies were conducted in accordance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals. The experiments with intravenous leptin were conducted first and the experiments with subcutaneous leptin administration were conducted in the same animals at least 1 week later. For both studies, the animals were fasted overnight and placed in restraint chairs 1 h before the experiment. A cephalic vein was catheterized for intravenous injection of leptin and for collection of blood samples. Two baseline blood samples were drawn from the venous catheter. Recombinant methuman leptin (Amgen Inc., Thousand Oaks, CA) was then administered rapidly intravenously or given as a subcutaneous injection in the interscapular region (both at 1 mg/kg body weight dissolved in saline). Thereafter, blood samples were taken at 2, 5, 15, 30, 60, 90, 120, 180, 240, 300 and 360 min after dosing while the animals were in the restraint chairs. The animals were returned to their home cages after 360 min. The 480 min sample was collected by venipuncture while the animals remained caged. One of the nine monkeys administered leptin intravenously failed to demonstrate a log–linear elimination phase in the 480 min sampling period, precluding our ability to accurately determine rate constants from this animal. Therefore, when calculating the pharmacokinetic characteristics, only eight animals were used in the intravenous administration series.

Mouse study

Female C57BL/6J mice were obtained from Bomholtgaard Breeding and Research Centre, Ry, Denmark, at 4 weeks of age. One week after arrival to the Experimental Department at Lund University, Malmö, mice were given either a high-fat diet ($n = 8$) or an ordinary rodent chow diet ($n = 6$; Research Diets, N Brunswick, NJ, USA). On a caloric base, the high-fat diet consisted of 16.4% protein, 25.6% carbohydrates and 58.0% fat (total 23.4 kJ/g), whereas the control diet consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat (total 12.6 kJ/g). Throughout the study period, the mice had free access to food and water. After 6 months, body weight was 24.5 ± 1.2 g in mice fed a normal chow and 32.9 ± 3.6 g in mice fed a high-fat diet. At this time, recombinant human leptin (Amgen Inc., Thousand Oaks, CA) was injected intraperitoneally at a dose level of 10 µg/g body weight in a volume of 10 µl/g body weight. Blood samples were taken from the retro-orbital venous plexus at 15, 60, 120, 180, 360 and 480 min after. Plasma was immediately separated following a centrifugation at 4°C and stored at −20°C until analysis. The study was approved by the Animal Ethics Committee at Lund University.

Leptin analysis

In the monkey study, plasma levels of human leptin were determined with a radioimmunoassay for primate leptin (Linco Research Inc., St Charles, MO, USA). The method uses a polyclonal rabbit antibody raised against recombinant human leptin, ^{125}I-labeled tracer prepared with recombinant human leptin and human leptin as standard. Compared with the standard Linco human leptin assay,[27] the primate leptin assay has been demonstrated to display 100% cross-reactivity with recombinant and endogenous human leptin. Rabbit anti-rabbit IgG was used for separation of bound and free leptin (Linco). In the mouse study, pre-treatment plasma leptin levels were determined with a radioimmunoassay specific for murine leptin (Ahrén *et al*,[23] Linco Research Inc., St Charles, MO). The method uses a polyclonal rabbit antibody raised against recombinant murine leptin, ^{125}I-labeled tracer prepared with recombinant mouse leptin and mouse leptin as standard. Rabbit anti-rabbit IgG was used for separation of bound and free leptin. The Linco human leptin assay[27] was used to measure leptin in mouse

plasma samples collected after the administration of recombinant human leptin.

Calculations and statistics

Pharmacokinetic analysis of the leptin concentration *vs* time data was performed using a nonlinear least-squares regression program (WinNonlin, Scientific Consulting Inc., Apex, NC) with weighting of the data proportional to $1/C_p$, where C_p is the plasma leptin level. Pharmacokinetic parameters were derived using the methods described by Gibaldi.[28] The equations $C_p = C_0 e^{-k10t}$ and $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ were used to describe a one-compartment and two-compartment open model, respectively. Volumes of distribution were calculated as follows: $V_d = \text{dose}/(A+B)$, $V_{AUC} = \text{dose}/(\beta * AUC_{inf})$, and for intraperitoneally administered leptin $V/f = \text{dose}/(k_{10} * AUC_{inf})$. Since the extent of intraperitoneal absorption could not be determined without repeating the mouse experiment with intravenous leptin administration, clearance (Cl) and volume of distribution (V) are reported as Cl/f and V/f, with f representing the fraction absorbed. For noncompartmental analysis the terminal log−linear phase was extrapolated to infinity to determine the area under the first moment curve ($AUMC_{inf}$). Data are reported as mean ± s.d. Differences between high-fat fed and normal-diet controls were evaluated using the two-sample Student t-test.

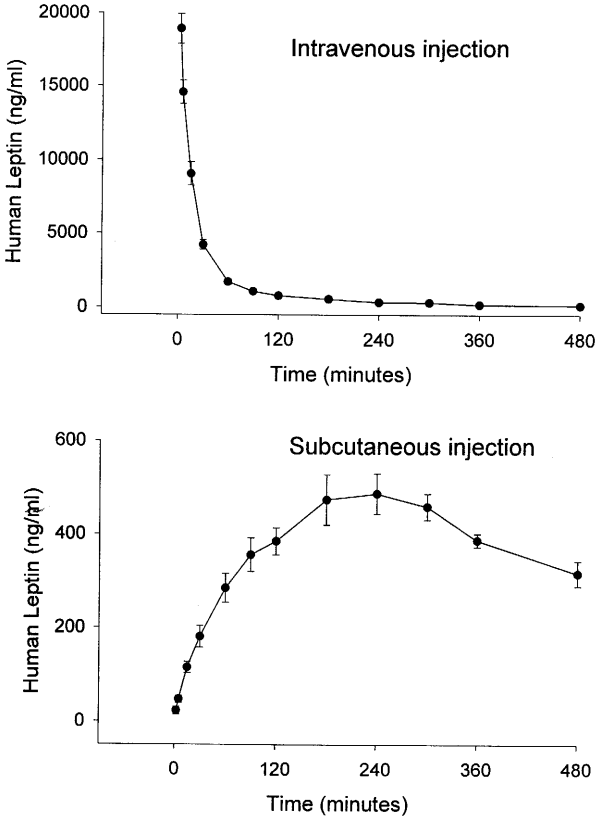

Figure 1 Plasma concentrations of human leptin before and after the intravenous (upper panel; $n=8$) or subcutaneous (lower panel; $n=9$) injection of recombinant human leptin (1 mg/kg) in male rhesus monkeys. Means ± s.e.m. are shown.

Results

Monkey study

Disposition of recombinant human leptin in monkeys following intravenous administration (Figure 1, upper panel) is characterized by a two compartment open model with a distribution phase ($t_{1/2}(\alpha) = 10.4 \pm 1.8$ min) and a slower elimination phase ($t_{1/2}(\beta) = 96.4 \pm 16.5$ min). The calculated pharmacokinetic constants are reported in Table 1. The kinetics of circulating leptin following subcutaneous injection are shown in the lower panel of Figure 1. Given the slow rate of absorption, the sampling period was not of sufficient length to obtain points during the log−linear elimination phase and hence the C_{max} (peak concentration) and T_{max} (time to peak concentration) were the only calculated parameters. Plasma leptin increased from a pre-injection concentration of 7.6 ± 5.3 ng/ml to a peak value of 544 ± 43 ng/ml. From visual inspection of the dataset, C_{max} values ranged from 378 to 712 ng/ml with T_{max} values occurring from 90 to 480 min post-injection.

Table 1 Pharmacokinetic characteristics of recombinant met-human leptin administered intravenously (1 mg/kg body weight) to monkeys ($n=8$)

Parameter	*Units*	*Mean ± s.d.*
Body weight	kg	12.0 ± 2.4
α	min^{-1}	0.068 ± 0.011
$t_{1/2}(\alpha)$	min	10.4 ± 1.8
β	min^{-1}	0.007 ± 0.001
$t_{1/2}(\beta)$	min	96.4 ± 16.5
AUC_{INF}	μg∗min/ml	554 ± 61.5
$AUMC_{INF}$	mg∗min^2/ml	42.6 ± 8.8
Clearance	ml/min/kg	1.82 ± 0.20
V_d	ml/kg	50.6 ± 9.2
V_{AUC}	ml/kg	253.8 ± 52.8
V_{ss}	ml/kg	139.8 ± 25.2
MRT_{INF}	min	76.7 ± 11.3

AUC = area under the curve; AUMC = area under the first moment curve; MRT = mean residence time. Means ± s.d. are shown.

Mouse study

Following intraperitoneal injection, plasma leptin concentrations in mice were best described by a one compartment open model with first order absorption and elimination (Figure 2). Mean pharmacokinetic values for mice fed either a control or a high fat diet are reported in Table 2. The absorption rate constant k_{01} and $t_{1/2}$ (k_{01}) are not reported because the sampling of mouse blood was focused on accurately determining leptin elimination kinetics, and hence the absorption phase was only partially defined. With the exception of the higher baseline levels of mouse leptin in mice fed a high-fat diet, the parameters were not significantly altered by diet. Plasma clearance and the elimination half-life in high-fat fed mice were 10.7 ± 1.4 ml/min/kg and 47.0 ± 26.4 min, respectively. Values for normal diet controls were 10.6 ± 1.4 ml/min/kg and 49.5 ± 12.0 min, respectively.

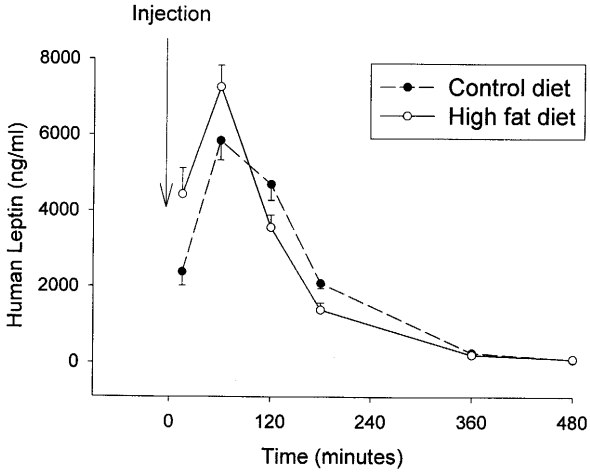

Figure 2 Plasma concentrations of human leptin after the intraperitoneal injection of recombinant human leptin (10 μg/g) in C57BL/6J mice fed a normal chow diet ($n=6$) or a high-fat diet ($n=8$) for 6 months. Means ± s.e.m. are shown.

Discussion

In this study, we observed biphasic kinetics following intravenous administration of recombinant human leptin to rhesus monkeys. The half-lives for the distribution phase $t_{1/2}$ (α) and the terminal elimination phase $t_{1/2}(\beta)$ were 10.4 ± 1.8 min and 96.4 ± 16.5 min, respectively, and the mean residence time was 76.7 ± 11.3 min. To our knowledge these results provide the first report of the pharmacokinetics of leptin in nonhuman primates. Comparison of our pharmacokinetic data with other studies using rodents or humans is difficult in some cases since a number of different methods to describe leptin kinetics have been employed. For example, a study in humans using an arteriovenous balance approach to determine endogenous leptin production on a per kg body fat basis, reported a leptin plasma half-life of 25 min and clearance of 1.5 ml/min/kg.[11] As the authors state, these values were considered estimates, as total body fat in the subjects was determined allometrically and the volume of distribution of leptin was assumed to be the calculated plasma volume.

The most readily comparable studies modeling the kinetics of exogenous leptin have been performed in rats where it was administered intravenously (Table 3). Using Sprague–Dawley rats, Cumin *et al*[29] administered three different doses of leptin and sampled blood for 3 h. Although their data best fit a three-compartment model, for all three doses the pharmacokinetic constants, V_d, MRT, and the terminal phase elimination half-life were all close to both one another and to our study. In another study conducted in rats, using a combination of arteriovenous balance and intravenous administration, Zeng *et al* found that leptin displayed biphasic kinetics.[30] When these data were reanalyzed and modeled using the same approach employed in the current study, the calculated half-lives and mean residence times, although slightly less, were comparable to our results (Landt unpublished results). Similar values were obtained by Hill *et al* using a dose that was approximately 1/1000 of those used in the current study and in the study by Cumin *et al*.[31] The shorter calculated half-lives observed in these studies may simply be attributable

Table 2 Pharmacokinetic characteristics of recombinant met-human leptin when administered intraperitoneally (10 μg/g body weight) to C57BL/6J mice fed a normal diet ($n=6$) or a high-fat diet ($n=8$)

Parameter	*Units*	*Normal diet*	*High-fat fed*	*P*
Body weight	g	24.5 ± 1.2	32.9 ± 3.6	< 0.001
k_{10}	min^{-1}	0.015 ± 0.003	0.018 ± 0.006	0.2832
$t_{1/2}(k_{10})$	min	49.5 ± 12.0	47.0 ± 26.4	0.8378
V/f	ml/kg	756 ± 221	702 ± 345	0.7424
Clearance	ml/min/kg	10.6 ± 1.4	10.7 ± 1.4	0.8986
AUC_{inf}	ng*min/ml	961246 ± 123393	953157 ± 122953	0.9063
$AUMC_{inf}$	ng*min^2/ml	118809267 ± 16203052	102161322 ± 30313745	0.2483
MRT_{inf}	min	123.6 ± 7.5	106.2 ± 23.0	0.1018
Baseline Leptin	ng/ml	10.4 ± 3.3	42.9 ± 23.6	0.0062

AUC = area under the curve; AUMC = area under the first moment curve; MRT = mean residence time.
Means ± s.d. are shown. *P* indicates the probability level of random difference between the groups.

Table 3 Pharmacokinetics of leptin as found in different studies in the literature

Reference	*Species*	*Route*	*Dose (mg/kg)*	*Time points (h)*	*α $t_{1/2}$ (min)*	*Terminal phase $t_{1/2}$ (min)*	*V_d (ml/kg)*	*V_{AUC} (ml/kg)*	*V_{ss} (ml/kg)*	*Cl (ml/min/kg)*	*MRT_{IV} (min)*
Present work	Monkey	i.v.	1.0	0–8	10.4±1.8	96.4±16.5	50.6±9.2	253.8±52.8	139.8±25.2	1.82±0.20	76.7±11.3
29	Rat	i.v.	0.25	0–3 h	1.5/7.2	90.0	58.12±1.74	*747*[a]	427.3±51.9	5.75±0.70	*75*[a]
		i.v.	0.5	0–3 h	1.2/6.0	87.0	58.12±8.48	*893*[a]	505.2±31.9	7.11±0.61	*71*[a]
		i.v.	1.0	0–3 h	1.7/7.2	91.8	66.0±9.47	*933*[a]	472.7±219	7.04±1.60	*70*[a]
31	Rat	i.v.	≈20 ng[c]	0–1 h	3.4	71	184.5	*631*[a]	—	6.16	—
30[b]	Rat	i.v.	3.5 ng	0–1.5 h	4.9 and 5.3	48 and 56	—	—	—	—	61 and 67
								V/f		*Cl/f*	MRT_{IP}
Present work	Mice/normal	i.p.	10	0–8 h	NA	49.0±11.7	—	754±219	—	10.6±1.4	122.3±7.7
Present work	Mice/fat	i.p.	10	0–8 h	NA	42.0±22.6	—	651±316	—	11.0±1.4	99.6±22.1

[a]Denotes value calculated from published results using: $MRT = (AUC_{INF} * V_{ss})/dose$, $V_{AUC} = Cl/\beta$.
[b]Values obtained from analysis of pharmacokinetic data kindly shared with us by Dr Michael Landt. Values were determined from two representative rats.
[c]Rats given a fixed amount of leptin. For a 300 g rat dose ≈0.0007 mg/kg.
Values are reported as mean±s.d.

to a shorter sampling period, and hence the terminal elimination phase was defined by a minimal number of later time points.

It is clear from comparing several different studies, using doses ranging over at least three orders of magnitude, that the rates of elimination and mean residence times are similar across species. The most striking difference observed in the kinetics of leptin in monkeys was a 68–74% lower plasma clearance compared to the fairly consistent values obtained in rats. It is noteworthy that clearance of 1.8 ml/min/kg obtained in the monkeys is similar to the value of 1.5 ml/min/kg reported in the human arteriovenous balance study.[11] Several groups have shown approximately 80% of whole body leptin clearance in humans and rats can be attributed to renal elimination.[30–33] However, the relative contributions of glomerular filtration and secretion to renal leptin clearance have been the subject of debate. Meyer *et al* reported an inverse relationship between renal fraction leptin extraction and circulating leptin level, and have suggested that renal uptake is a saturable process modelled by Michaelis–Menton kinetics.[33] It is difficult to reconcile these findings with our results which demonstrate that plasma clearance is virtually the same in monkeys as the value reported in humans, when plasma leptin levels in the monkeys were 1–2 orders of magnitude higher.

Since clearance, rate of elimination and volume of distribution are all directly related, V_{AUC} and V_{SS}, were both reduced to a similar degree as clearance, despite little differences in the initial volume of distribution across species. Although significantly less compared to rodents, the V_{SS} of 140 ml/kg or 13.9% of body mass in monkeys, indicates that leptin most likely distributes beyond the plasma volume and throughout the extracellular space. As the leptin assays employed are considered to measure both bound and free leptin, we cannot make any inferences on the extent of plasma protein binding. Nonetheless, these findings provide some of the first information regarding the kinetics of exogenous leptin in non human primates, and the results may be applicable to humans.

Our failure to detect the log–linear elimination phase within the 8 h sampling period following subcutaneous administration suggests that the rate of leptin absorption after subcutaneous administration is quite slow, perhaps saturated. Zero order absorption kinetics could potentially explain the near plateau observed in Figure 1. This may have clinical significance as route of administration and formulation are considered for human administration.

As shown in previous studies, high fat fed mice were found to have significantly increased baseline circulating leptin levels.[23–25] The mean baseline plasma leptin concentration for high fat fed mice was 43±24 ng/ml compared to 10±3 ng/ml in the mice consuming the control diet. In contrast, there was no statistical difference in any of the determined pharmacokinetic parameters between the two groups

of mice. Similarly, Klein *et al* found no differences in leptin plasma clearance or half-life between normal weight and obese human subjects.[11] A study in obese and lean Zucker rats also found no statistical difference in kinetics following intravenous administration.[21] However, in that study, blood was only collected during the first eight minutes after injection and therefore little conclusions on the terminal elimination phase could be drawn.

The steady-state level of endogenous leptin is equal to the rate of whole body leptin production (LP) divided by the clearance ($C_{SS} = LP/Cl$). Given that clearance did not differ between the two groups of mice, and assuming a similar fraction absorbed, the four-fold higher base-line leptin levels in high-fat fed mice must therefore be explained by an increase in whole body leptin production of roughly the same magnitude. A similar conclusion was made by Klein *et al* based on their studies with obese humans.[11] The mean volumes of distribution observed in mice, 756 ml/kg in mice fed a normal diet and 702 ml/kg in mice fed a high-fat diet indicate that leptin distributes into a much larger space than plasma volume. Whether the large volume is attributable simply to widespread distribution, or a significant contribution is due to leptin binding to other proteins in the extravascular space remains unknown. Compared to monkeys, mice have a volume of distribution approximately three times larger and clear leptin six times faster, although a caveat is that these values will be affected by the fraction of leptin absorbed, as leptin was administered by the intraperitoneal route in mice. Nevertheless, even if absorption was incomplete, a large difference in leptin kinetics would still exist in mice compared with monkeys.

In conclusion, the results of these experiments indicate that primates and rodents display similar terminal elimination half-lives and mean residence times for intravenously administered leptin. In contrast, clearance and the volume of distribution were significantly different between the two species. It should be emphasized that our conclusions are valid for the pharmacokinetic characteristics of human leptin in monkeys and mice. However, whether these characteristics differ from species-specific leptin remains to be established. Nonetheless, marked differences in the pharmacokinetics of human leptin between humans and rhesus monkeys appear to be unlikely considering that the clearance of human leptin in monkeys is similar to values previously observed in humans. Our results, together with those from previously published studies,[29–31] indicate that the clearance of leptin does not appear to be a saturated process at any point over the wide range of doses employed in rats, primates and humans. In addition, leptin administered extravascularly in saline is absorbed slowly, with subcutaneous absorption appearing near zero-order. The present study also found that there are no statistically significant differences in the pharmacokinetics of exogenous leptin between normal weight chow-fed mice and mice made obese by high-fat feeding, suggesting that steady-state leptin levels in obesity do not result from a decreased rate of elimination, but from an increased rate of production and secretion.

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