Adipose Tissue Leptin Production and Plasma Leptin Kinetics in Humans

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Abdominal adipose tissue leptin production was determined in vivo by arteriovenous balance in 14 lean and obese men (mean BMI 27.0 ± 1.9, range 21.4–45.2). Blood samples were taken simultaneously from an abdominal vein that drains subcutaneous adipose tissue and from a radial artery. Adipose tissue blood flow was measured by xenon washout. Abdominal vein leptin concentrations (mean 8.9 ± 2.4 ng/ml, range 2.1–36.5 ng/ml) were consistently greater than arterial values (mean 6.6 ± 1.9 ng/ml, range 1.7–28.2 ng/ml) ($P < 0.001$). The net rate of abdominal adipose tissue leptin production (mean 3.2 ± 0.5 ng · 100 g^{-1} · min^{-1}) correlated directly with percentage body fat ($r_s = 0.59$, $P = 0.016$). Estimated whole-body leptin production rate (797 ± 283 ng · $person^{-1}$ · min^{-1}) correlated directly with percent body fat ($r_s = 0.93$, $P < 0.0001$) and with regional leptin production ($r_s = 0.81$, $P < 0.001$). In contrast, the rate of leptin clearance from plasma (mean 1.50 ± 0.23 ml · kg^{-1} · min^{-1}) and plasma leptin half-life (mean 24.9 ± 4.4 min) was unrelated to adiposity ($r_s = 0.06$, $P = 0.30$; $r_s = 0.16$, $P = 0.30$, respectively). These results provide direct evidence that leptin is produced by adipose tissue in humans and that the rate of production is directly related to adiposity. A combination of greater leptin production per unit of body fat and increased production from expanded total body fat mass, rather than alterations in leptin clearance, account for the increase in plasma leptin concentrations observed in obese humans. *Diabetes* 45:984-987, 1996

The discovery of the obese (*ob*) gene and its protein product, leptin, represents an important development in our understanding of the pathophysiology of obesity. In *ob/ob* mice, a mutation in the *ob* gene prevents the production of leptin and thus causes hyperphagia, inactivity, and ultimately obesity. Treatment of *ob/ob* mice with exogenous leptin decreases food intake and increases physical activity, which leads to a decrease in body mass (1–3). In contrast to what is seen in *ob/ob* mice, leptin production in most obese humans does not seem to be impaired because circulating leptin concentrations increase with BMI and body fat content (4,5). However, these conclusions are based on static measurements of serum leptin concentration, which may not represent true differences in leptin production rates. Better understanding of leptin production rates and other aspects of leptin kinetics would provide further insights into the relationship between leptin metabolism and obesity in humans.

In the present study, we evaluated leptin kinetics in vivo in lean and obese humans. Net basal adipose tissue leptin production rates were determined by using standard arteriovenous balance principles. A superficial abdominal vein that drains subcutaneous abdominal adipose tissue and a peripheral artery were cannulated in each subject to measure simultaneous arterial and venous serum leptin concentrations. Abdominal adipose tissue blood flow was measured by the xenon washout technique. Regional leptin production rate was used to estimate whole-body leptin production rate, plasma leptin clearance, plasma leptin pool fractional turnover rate, and leptin half-life.

RESEARCH DESIGN AND METHODS

Subjects. A total of 14 male volunteers participated in this study (Table 1). All subjects were weight-stable at the time of the study and had no evidence of medical diseases after completing a comprehensive medical evaluation, including history and physical examination, blood tests, and an electrocardiogram.

The study was approved by the Human Studies Committee of Washington University School of Medicine and University College London Medical School. Informed written consent was obtained from all subjects before their participation.

Study protocol. Subjects were admitted to the General Clinical Research Center on the evening before each study and given a standard meal. After the subjects fasted overnight (12 h), a 20-gauge catheter was inserted into a radial artery at the wrist to obtain arterial blood samples. An abdominal vein draining subcutaneous abdominal adipose tissue was cannulated by inserting a 10-cm 22-gauge polyurethane catheter (Hydrocath, Viggo-Spectramed, Oxnard, CA) over a guide wire using the Seldinger technique. The catheter was positioned so that the tip was superior to the inguinal ligament (judged by surface anatomy). In this position, blood withdrawn from the catheter represents drainage from adipose tissue and overlying skin without contribution from underlying muscle (6). Basal arterial and abdominal venous blood samples were obtained simultaneously at 60, 90, and 120 min after placement of both catheters. Abdominal subcutaneous adipose tissue blood flow (ATBF) was measured using the ^{133}Xe washout technique based on the principle that the disappearance of ^{133}Xe radioactivity is proportional to ATBF (7). Immediately after placement of the abdominal vein catheter, ~150 μCi of ^{133}Xe dissolved in 0.1 ml of saline was slowly injected over 2 min into the subcutaneous abdominal adipose tissue space. The decline in ^{133}Xe was monitored from 60 to 120 min after injection with a 2-inch sodium iodide scintillation detector. The detector was placed ~40 cm from the ^{133}Xe depot and was coupled with a multichannel analyzer set to measure the 81-keV ^{133}Xe photopeak.

Analysis of samples. Arterial and venous leptin plasma concentrations were determined by a newly developed radioimmunoassay using a polyclonal antibody raised in rabbits against highly purified recombinant human leptin (8). Briefly, samples, calibrators, and quality control media were incubated with ^{125}I-labeled recombinant human leptin and then with rabbit antibody to recombinant human leptin (Linco Research, St. Louis, MO). After 18 h, antibody-bound ^{125}I-leptin was precipitated by addition of anti-rabbit IgG, and the precipitates were collected by

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ATBF, adipose tissue blood flow; ATPF, adipose tissue plasma flow.

TABLE 1
Characteristics of the study subjects

Characteristic	Mean ± SE	Range
Age (years)	45 ± 3	22–70
Height (cm)	174 ± 2	158–187
Weight (kg)	81 ± 5	65–117
BMI (kg/m^2)	27.0 ± 1.9	21.4–45.2
Body fat (%)	23.3 ± 2.8	13.3–48.2

For study subjects, n = 14.

centrifugation. Precipitated counts per minute were related to sample leptin concentration by logit-log transformation of counts for calibrators (recombinant human leptin) ranging from 0.5 to 100 ng/ml, corresponding to the limit of detection and linear range of the assay, respectively. The coefficient of variation for within- and between-run analyses ranged from 3.4 to 8.3% and from 3.6 to 6.2%, respectively. Plasma cortisol concentrations were determined with a commercial fluorescence polarization assay (TDx, Abbott Laboratories, Abbott Park, IL).

Calculations. Subcutaneous ATBF was calculated from ^{133}Xe clearance (7).

$$\text{ATBF (ml} \cdot 100 \text{ g adipose tissue}^{-1} \cdot \text{min}^{-1}) = -k\lambda \cdot 100 \text{ (ml} \cdot 100 \text{ g adipose tissue}^{-1} \cdot \text{min}^{-1})$$

where k is the rate constant of the ^{133}Xe monoexponential washout curve and λ is the adipose tissue–to–blood partition coefficient for xenon. The values for k were determined experimentally as $(\ln y_2 - \ln y_1)/60$, where y_1 and y_2 were the counting rates at times 60 and 120 min, respectively. The value for λ was assumed to be 10 ml/g (7). Adipose tissue plasma flow (ATPF) was calculated as ATBF · (1 hematocrit).

Regional subcutaneous abdominal adipose tissue net leptin production rate was calculated using standard principles of arteriovenous balance (9).

$$\text{Adipose tissue leptin production (ng} \cdot 100 \text{ g adipose tissue}^{-1} \cdot \text{min}^{-1}) = \text{ATPF } ([L_a] - [L_v])$$

where ATPF is the rate of subcutaneous adipose tissue plasma flow in ml · 100 g adipose tissue^{-1} · min^{-1}, $[L_a]$ is the arterial concentration of leptin in nanograms per milliliter, and $[L_v]$ is the venous concentration of leptin.

An estimate of whole-body net leptin production rate was made by using a predictive equation, based on BMI, sex, and age, to calculate total body fat mass (10) and by multiplying adipose tissue leptin production rate by total body fat mass.

The rate of leptin clearance from plasma was calculated by dividing whole-body net leptin production in nanograms per kilogram body weight per minute by $[L_a]$ in nanograms per milliliter.

Plasma fractional leptin turnover, k, represents the fraction of the plasma leptin pool that leaves the plasma compartment per minute and was calculated by dividing whole-body net leptin production in nanograms per person per minute by the plasma leptin pool size in nanograms. Plasma leptin pool size in milliliters was determined by multiplying plasma volume, estimated to be 37 times body weight in kilograms (11), by $[L_a]$.

Plasma leptin half life ($t_{1/2}$) is equal to ln 2/k and describes the time it takes leptin released into the circulation from adipose tissue to reach half of its original concentration.

Statistical analyses. Student's t test for paired samples (two-tailed) was used to test the significance of differences between arterial and venous samples. A simple linear regression was used to determine the relationship between variables, and the statistical significance of the relationships was evaluated by Spearman's rank-sum correlation (r_s). Because of the absence of subjects with BMI values between 30 and 40 kg/m^2, the regression data were analyzed with and without the two subjects with BMI >40 kg/m^2. A P value of <0.05 was considered to be statistically significant. All tabular data are expressed as means ± SE.

RESULTS

Plasma leptin concentrations. Basal arterial leptin concentrations ranged from 1.7 to 28.2 ng/ml (mean ± SE, 6.6 ± 1.9 ng/ml) and correlated directly with BMI (r_s = 0.95, P < 0.0001) and percentage body fat (r_s = 0.76, P = 0.002). Abdominal vein leptin concentrations ranged from 2.1 to 36.5 ng/ml (8.9 ± 2.4 ng/ml) and were consistently greater than the corresponding arterial values measured in the same subjects (P < 0.001). Although plasma leptin concentrations encompassed a large range, the relationship between venous and arterial values was similar among all subjects. Venous leptin concentrations were 140 ± 5% of those in arterial blood, demonstrating that plasma leptin increases substantially in a single pass across adipose tissue.

Plasma cortisol concentrations. Plasma cortisol concentrations were measured to serve as an internal control for the leptin values. In contrast to the differences observed between arterial and venous leptin concentrations, radial artery and abdominal vein cortisol concentrations were identical; mean arterial and venous cortisol concentrations were 8.37 ± 0.82 and 8.51 ± 0.75 μg/dl, respectively. The ratio of arterial to venous values was 0.97 ± 0.02. The cortisol data demonstrate that the observed differences between arterial and venous leptin concentrations were not caused by arterial or venous fluid shifts.

Blood flow. ATBF ranged from 1.22 to 8.64 ml · 100 g^{-1} · min^{-1} (3.19 ± 0.60 ml · 100 g^{-1} · min^{-1}) and was inversely correlated with BMI (r_s = −0.55, P = 0.04). ATPF ranged

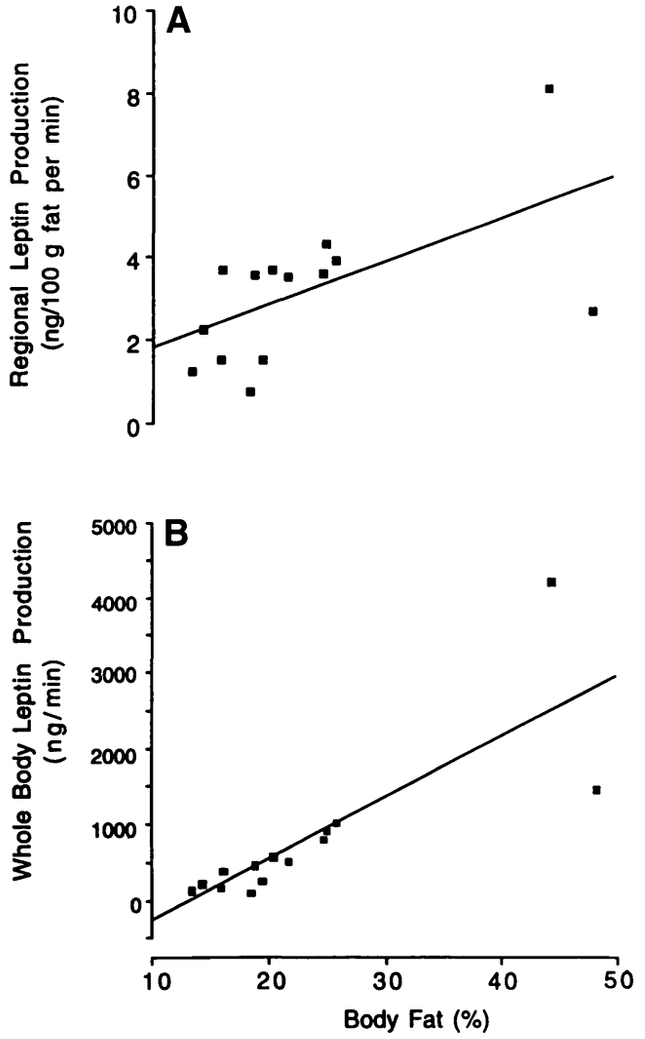

FIG. 1. Relationship between plasma leptin kinetics and adiposity. Abdominal subcutaneous adipose tissue leptin production (*A*) was directly correlated with percentage body fat (r_s = 0.59; P = 0.016). Estimated whole-body leptin production rate (*B*) was also directly correlated with percentage body fat (r_s = 0.93; P < 0.0001).

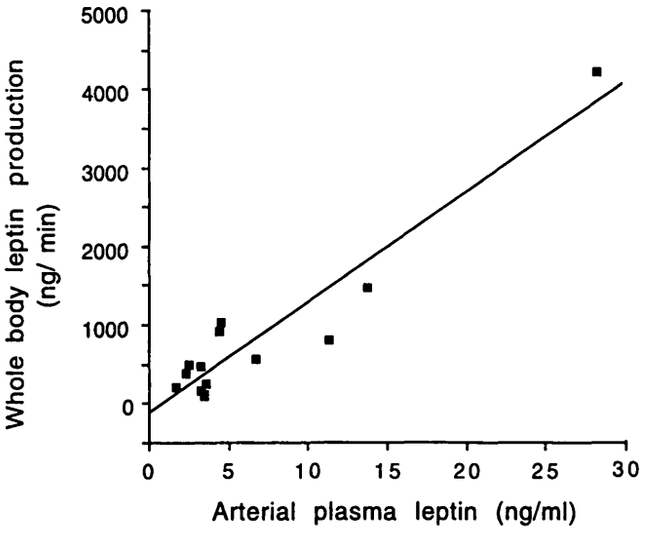

FIG. 2. Relationship between arterial plasma leptin concentration and estimated whole-body plasma leptin production. Arterial plasma leptin concentration correlated closely with whole-body plasma leptin production (r_s = 0.93; P < 0.0001).

from 0.73 to 5.18 ml · 100 g^{-1} · min^{-1} (1.91 ± 0.36 ml · 100 g^{-1} · min^{-1}) and was also inversely correlated with BMI (r_s = −0.58, P = 0.03).

Leptin kinetics. Leptin production rates were associated with adiposity. Abdominal adipose tissue net leptin production rate was 3.18 ± 0.49 ng · 100 g^{-1} · min^{-1}, and whole-body leptin production rate was 797 ± 283 ng · $person^{-1}$ · min^{-1}. Regional net leptin production correlated directly with percentage body fat (r_s = 0.59, P = 0.016) (Fig. 1). Estimated whole-body leptin production increased with percentage body fat (r_s = 0.93, P < 0.0001) (Fig. 1) and with regional net leptin production (r_s = 0.81, P < 0.001). Basal arterial plasma leptin concentration correlated closely with whole-body leptin production (r_s = 0.93, P < 0.001) but not with regional leptin production (r_s = 0.39, P = 0.17) (Fig. 2).

Removal of leptin from plasma was not related to adiposity. Plasma leptin clearance, fractional plasma leptin turnover, and plasma leptin half-life were 1.50 ± 0.23 ml · kg^{-1} · min^{-1}, 4 ± 1% of plasma pool per minute, and 24.9 ± 4.4 min, respectively. No significant correlations were detected between percentage body fat and plasma leptin clearance rate (r_s = 0.06, P = 0.30), fractional plasma leptin turnover (r_s = 0.05, P = 0.30), or plasma leptin half-life (r_s = 0.16, P = 0.30) (Fig. 3).

Linear relationships were also determined in the 12 subjects who had BMI values <40 kg/m^2 to exclude the two heaviest subjects, who might be considered outliers (data not shown). Regression analyses for these 12 subjects were similar to the data obtained from the entire group and did not change any of the statistically significant relationships. Residual values for all reported regression analyses were randomly distributed and did not correlate with any independent variables.

DISCUSSION

In the present study, we evaluated plasma leptin kinetics in humans by measuring net leptin balance across abdominal adipose tissue. Our data demonstrate, for the first time, that adipose tissue produces leptin that is then secreted into the systemic circulation. In addition, leptin production was not

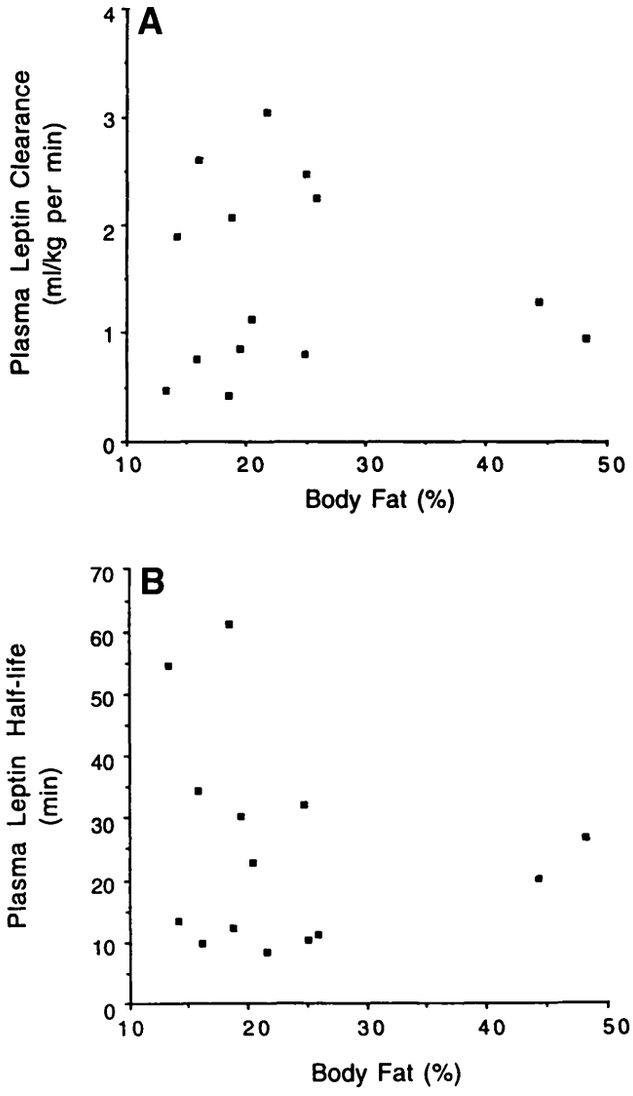

FIG. 3. Relationship between plasma leptin clearance and adiposity (*A*) and between plasma leptin half-life and adiposity (*B*). Neither leptin clearance (r_s = 0.06; P = 0.30) nor leptin half-life (r_s = 0.16; P = 0.30) was related to percentage body fat.

impaired in obese humans; in fact, whole-body leptin production rates correlated directly with BMI and percentage body fat mass. In contrast, the rate of leptin clearance from plasma was independent of body mass and adiposity. Therefore, the increase in plasma leptin concentration associated with increasing body fat mass in the present study and reported previously (4,5,8) is due to an increase in whole-body leptin production, not a defect in leptin clearance. These data provide further evidence that obesity in humans is not caused by abnormal protein production by the obese gene.

The increase in regional leptin appearance with increasing BMI observed in our subjects suggests that increased adiposity causes upregulation of leptin production per unit of fat mass. This finding is consistent with data obtained in vitro from isolated adipocytes that demonstrated overexpression of the obese gene in obese subjects (4,12–14). Therefore, both increased total body fat mass and upregulation of leptin production per unit of fat are responsible for the overall increase in leptin production in obese humans. However, the relative contribution of each variable to plasma leptin concentrations cannot be determined from our data because of the insufficient number of subjects.

The calculated mean plasma half-life of leptin in our subjects was 25 min and is within the range of values reported for other protein hormones. The plasma half-lives of small peptide hormones, such as the neurohypophysial, gastrointestinal, and pancreatic hormones, are between 2 and 10 min, whereas the plasma half-life of growth hormone, which has a similar polypeptide size to leptin, is between 20 and 30 min (15). The similar half-lives of leptin and growth hormone suggests that their larger sizes may confer some degree of metabolic stability in comparison with smaller peptide hormones.

The plasma half-life of leptin seems surprisingly short if it is regulated solely by body fat mass, because changes in body fat mass occur over days to weeks. However, the estimate of leptin plasma half-life in our subjects is consistent with data demonstrating that plasma leptin concentrations change in relationship to recent (hours to days) energy intake before significant changes in body composition occur (4). Other factors that might affect short-term leptin production include recent energy balance (14,16), insulin sensitivity (16,17), sympathetic nervous system activity (18), and corticosteroids (19).

The rate of whole-body leptin production calculated in our subjects required making several assumptions, including an estimate of body composition and an assumption of leptin production homogeneity among all adipose tissue depots. Errors of a magnitude that might reasonably occur in our estimations would not qualitatively change any of our conclusions. For example, an error in estimating body fat by several kilograms would alter whole-body leptin production rates by only a fraction of the current estimate and hence estimated plasma half-life by only a few minutes. The impact of potential regional differences in leptin production rates is more difficult to assess. In one study, obese gene expression was different in subcutaneous adipose tissue compared with intraperitoneal sites (20), while in another there was no difference between subcutaneous and intraperitoneal fat (13). We calculated whole-body leptin kinetics by extrapolating data obtained from a single subcutaneous adipose tissue depot. The predominance of subcutaneous fat, which accounts for >80% of total body fat (21), would lessen the impact of any heterogeneity between subcutaneous and other adipose tissue sites and could account for the strong correlation of our estimate of whole-body leptin production rate with arterial leptin concentration. It is also possible that leptin production might differ among different subcutaneous adipose tissue sites. Other in vivo metabolic activities, such as lipolysis, have been reported to be the same (22) or to differ by as much as 30% (23) in lower compared with upper subcutaneous adipose tissue sites. Therefore, it is unlikely that our conclusions would be altered by regional differences in leptin production, provided the potential differences were similar to those reported for lipolysis.

In summary, the present study provides direct evidence that leptin is produced by adipose tissue in humans and the rate of production is directly related to adiposity. Both greater leptin production per unit of body fat and increased total body fat mass, rather than alterations in leptin clearance, account for the increase in plasma leptin concentrations observed with increasing adiposity in humans.

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