

## Extra-adipocyte leptin release in human obesity and its relation to sympathoadrenal function

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**Eikelis, Nina, Gavin Lambert, Glen Wiesner, David Kaye, Markus Schlaich, Margaret Morris, Jacqueline Hastings, Florentia Socratous, and Murray Esler.** Extra-adipocyte leptin release in human obesity and its relation to sympathoadrenal function. *Am J Physiol Endocrinol Metab* 286: E744–E752, 2004. First published January 13, 2004; 10.1152/ajpendo.00489.2003.—The link between the human sympathoadrenalmedullary system and the adipocyte hormone leptin is controversial. We measured total and regional norepinephrine spillover, epinephrine secretion rate, and extra-adipocyte leptin release in 22 lean [body mass index (BMI) <26] and 20 obese (BMI >28) normotensive men who underwent arterial and central venous catheterization. Because plasma clearance of leptin is primarily by renal removal, for men at steady state we could estimate whole body leptin release to plasma from renal plasma leptin extraction. Whole body leptin release was  $1,950 \pm 643$  (means  $\pm$  SE) ng/min in obese men and  $382 \pm 124$  ng/min in lean men ( $P < 0.05$ ). Total and renal norepinephrine spillover rates correlated directly with whole body leptin secretion rate. Leptin is released from multiple nonadipocyte sites, which we tested by use of simultaneous arteriovenous blood sampling. We found a surprisingly large contribution of brain leptin release to the plasma leptin pool,  $529 \pm 175$  ng/min (>40% whole body leptin release), with greater leptin release in obese than in lean men,  $935 \pm 321$  vs.  $160 \pm 59$  ng/min ( $P = 0.045$ ). In parallel with leptin measurements, we also quantified brain serotonin turnover and jugular overflow of neuropeptide Y (NPY). Brain serotonin turnover was higher in obese than in lean men,  $227 \pm 112$  vs.  $21 \pm 14$  ng/min ( $P = 0.019$ ), as was overflow of NPY from the brain,  $12.9 \pm 1.4$  vs.  $5.3 \pm 2.2$  ng/min ( $P = 0.042$ ). These results suggest that leptin is released within the brain and at an increased rate in obese humans, in whom activation of brain serotonergic and NPY mechanisms also exists.

leptin; norepinephrine spillover; epinephrine secretion rate; 5-hydroxyindoleacetic acid; neuropeptide Y

THE PREVALENCE OF OBESITY is rising at an alarming rate worldwide, with consequent increases in type 2 diabetes, hypertension, and cardiovascular mortality. Despite this, the biological mechanisms predisposing humans to weight gain remain poorly understood.

On the basis of findings in animal models of obesity, the sympathoadrenal system has commonly been assumed to have a determining role in obesity development through its influence on regulation of energy expenditure. A widely held view (3, 33) has been that reduced sympathoadrenal activity, by leading to diminished thermogenesis, underlies the development of obesity. Although more recent evidence indicates that sympa-

thetic nervous activity is actually increased in human obesity (13), the case is still open for adrenal medullary secretion of epinephrine as an important contributor to the development of obesity (49).

The functions of the sympathoadrenal system and the adipocyte hormone leptin (50) appear to be intimately linked (5). In rodents, leptin promotes weight loss by reducing appetite and through increasing sympathetically mediated energy expenditure (43). Experimental evidence exists of a two-way interaction between leptin and the sympathetic nervous system, perhaps constituting a regulatory feedback loop, with leptin acting within the hypothalamus to cause activation of central sympathetic outflow and, conversely, with the sympathetic nervous system inhibiting leptin release from white adipose tissue (42), although recently we found the strength of this latter relation in humans to be weaker than anticipated (7).

At one time it was assumed that adipocytes were the sole site of leptin production and release. Evidence from studies in experimental animals and humans now indicates that leptin is also derived from nonadipose sites (1, 14, 29), one of these being the brain (14, 29). This putative brain leptin system remains very incompletely categorized, and possible relationships to other brain neuronal systems regulating feeding behavior and energy expenditure are largely unexplored. Neuropeptide Y (NPY) concentration is increased in the hypothalamus in some experimental genetic models of obesity (47), and NPY has been implicated in the effects of leptin on feeding behavior (36). Brain serotonergic mechanisms have also been linked experimentally with the central nervous system (CNS) actions of leptin (16, 48). The hypothalamus is highly innervated by serotonin-containing fibers, and the serotonergic system has been the target of a number of drugs aiming to regulate body weight (25).

In the present study, we have explored these issues further, concentrating in particular on the interplay between the sympathoadrenal system and leptin, and on brain leptin, NPY, and serotonin mechanisms in human obesity. We tested whether an association existed between rates of leptin secretion, which we estimated from leptin renal clearance rates, and regional sympathetic nervous activity. Whether reduced adrenal medullary secretion of epinephrine might be present in obesity, perhaps contributing to its development (24, 49), was tested by determining epinephrine secretion rates in lean and obese men by isotope dilution methodology (11). We used simultaneous arteriovenous blood sampling to quantify leptin release to plasma from individual organs, including internal jugular ve-

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nous blood sampling to test for leptin efflux from the brain (14). We also tested for NPY jugular venous overflow in obesity, which is not present in lean men (30), and quantified brain serotonin turnover from the rate of jugular overflow of the major metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) (21).

## METHODS

### Subject Characteristics

Twenty-two lean and 20 obese healthy male volunteers were recruited from the general community for the study. The lean men had a body mass index (BMI) of <26; the obese men had a BMI >28. The study was confined to men to avoid the confounding influence of gender on leptin values. All participants had thorough clinical evaluation and serum biochemistry measurements to exclude hepatic and renal dysfunction. All research volunteers were unmedicated, and those with a history of cardiovascular disease or diabetes, a blood pressure >140/85 mmHg, obstructive sleep apnea, or an alcohol intake of more than two standard drinks per day were excluded from the study. All subjects had been weight stable ( $\pm 1$  kg) for  $\geq 2$  mo before the study, and lean subjects did not have a prior history of obesity. Characteristics of the two groups are summarized in Table 1. Participation in the study was after written informed consent, with the approval of the Alfred Hospital Ethics Review Committee.

A separate subset of nine lean and five obese male volunteers with normal blood pressures was used to quantify jugular overflow of neuropeptide Y (NPY).

### Catheter Procedure

All studies were performed after an overnight fast. The subjects were asked to refrain from caffeinated beverages and alcohol for the 12 h preceding the catheter study. The catheter procedure was performed with the subjects in the supine position. In a research cardiac catheterization laboratory, a 21-gauge cannula was introduced percutaneously under local anesthesia into the brachial or radial artery of either arm for blood sampling. In the same arm, an 8.5-Fr gauge introducer sheath was inserted percutaneously into the median antecubital vein. Venous catheterization was performed via this sheath. The coronary sinus, renal vein, internal jugular vein, and hepatic vein were reached by maneuvering this single central catheter under direct fluoroscopic control. In the internal jugular vein, the catheter tip was above the angle of the jaw to exclude sampling from the tissues of the face. Correct positioning of the catheter was confirmed using 2-ml radiopaque contrast medium.

In all studies, 10-ml blood samples for measurement of the chemicals of interest were obtained simultaneously from the arterial and venous catheters and immediately placed into ice-chilled tubes con-

taining glutathione as antioxidant and EGTA as anticoagulant. At the completion of the catheter study and within 15–75 min of sampling, the blood samples were centrifuged at 4°C and the plasma was stored at –80°C until assayed.

### Plasma Catecholamine Assays

Plasma catecholamine concentrations were determined by high-performance liquid chromatography with electrochemical detection (HPLC) (28). Timed collection of the eluate leaving the detection cell with a fraction collector permitted separation of <sup>3</sup>H-labeled norepinephrine (NE) and epinephrine for counting by liquid scintillation spectroscopy. Intra-assay variations were 4.6% for plasma NE and 6.8% for plasma epinephrine at concentrations of 150 pg/ml and 7.2 and 6.5% for <sup>3</sup>H-labeled NE and epinephrine.

Whole body, renal, and cardiac NE spillover rates were determined as measures of sympathetic activity (11). Subjects were infused with levo-7-[<sup>3</sup>H]norepinephrine (New England Nuclear, Boston, MA), specific activity 11–25 Ci/mmol, at a rate of –0.6–0.8  $\mu$ Ci/min. At steady state, whole body NE spillover to plasma was determined from arterial sampling with isotope dilution. Epinephrine secretion rates were determined similarly (11), from arterial sampling during the infusion of levo-7-[<sup>3</sup>H]epinephrine (New England Nuclear), specific activity 69–78 Ci/mmol, at a rate of –0.6–0.8  $\mu$ Ci/min.

Regional NE spillover from the heart and kidneys was calculated from the venoarterial plasma NE concentration difference across the organ in question, the fractional extraction of radiolabeled NE occurring in transit, and regional plasma flow (11). Coronary sinus and internal jugular plasma flows were derived from thermodilution blood flow measurements and the hematocrit (12), renal plasma flow was determined from the clearance of infused *p*-aminohippuric acid, and hepatic blood flow was quantified from the clearance of indocyanine green.

### Plasma Leptin Assay

Total plasma leptin levels were measured in duplicate by radioimmunoassay (Linco Research, St. Charles, MO), with an intra-assay coefficient of variation of 5% and a sensitivity of 0.5 ng/ml. Renal leptin clearance was calculated as the product of the fractional extraction of plasma leptin in transit through the kidney and renal plasma flow. Whole body leptin secretion rate was calculated as the product of arterial plasma leptin concentration and renal leptin clearance. The rate of release of leptin from the brain was calculated from the product of the internal jugular-arterial venoarterial plasma leptin concentration difference and the internal jugular plasma flow.

Because clearance of leptin from plasma is almost exclusively by renal removal (4), by applying the principle of the conservation of mass we estimated the rate of whole body leptin release to plasma at steady state from measurements of renal leptin extraction from plasma in transit through the kidneys by use of renal vein sampling. Renal leptin extraction was derived from the product of the arterial plasma concentration of leptin and leptin renal plasma clearance.

### Plasma 5-HIAA Assay

The plasma concentration of the major serotonin metabolite 5-HIAA was determined by HPLC, as previously described (20). Brain serotonin turnover was calculated from the product of the internal jugular-arterial venoarterial plasma 5-HIAA concentration difference and the internal jugular plasma flow (21).

### Plasma NPY Assay

NPY-like immunoreactivity (NPY-LI) concentration was determined by radioimmunoassay of plasma samples and NPY standards, as previously described (30). Chromatographic characterization of the NPY-LI detected in human plasma was carried out by HPLC separa-

Table 1. Subject characteristics and measures of sympathetic nervous activity

	Lean (BMI <26)	Obese (BMI >28)
<i>n</i>	22	20
Age, yr	39.3 $\pm$ 4.3	47.3 $\pm$ 2.4
BMI, kg/m <sup>2</sup>	22.4 $\pm$ 0.4	31.0 $\pm$ 0.6*
Systolic BP, mmHg	139.0 $\pm$ 4.7	136.0 $\pm$ 3.6
Diastolic	73.0 $\pm$ 1.9	75.0 $\pm$ 1.3
Heart rate, beats/min	65.0 $\pm$ 2.6	63.0 $\pm$ 2.0
Arterial leptin concn, ng/ml	2.9 $\pm$ 0.3	10.2 $\pm$ 1.2*
NE spillover, ng/min		
Total	436.7 $\pm$ 48.2	450.4 $\pm$ 52.8
Renal	64.2 $\pm$ 13.0	150.3 $\pm$ 30.6*
Cardiac	20.7 $\pm$ 3.6	11.7 $\pm$ 2.9

Values are means  $\pm$  SE; *n*, no. / group. BP, blood pressure; BMI, body mass index; NE, norepinephrine. \**P* < 0.05; significant differences between groups.

tion with a linear acetonitrile gradient. The rate of release of NPY from the brain was calculated from the product of the internal jugular-arterial venoarterial plasma NPY concentration difference and the internal jugular plasma flow.

#### Statistical Analysis

Statistical analyses were performed with SigmaStat for Windows version 2.03 (Jandel Scientific, San Rafael, CA) and with a Student's *t*-test and ANOVA, where appropriate. If normality or equal variance failed, the Mann-Whitney rank sum test was employed. Data are expressed as means  $\pm$  SE. Significance was set at  $P < 0.05$ .

## RESULTS

### Epinephrine Plasma Kinetics in Obesity

No differences were found in epinephrine plasma kinetics values between lean and obese men. Epinephrine secretion rates were also similar in the two groups (Fig. 1).

### Regional Sympathetic Nervous System Activity in Obesity

Renal NE spillover, indicative of renal sympathetic activity (32), was significantly higher in obese ( $150.3 \pm 30.6$  ng/min) than in lean men ( $64.2 \pm 13.0$ ;  $P = 0.013$ ; Table 1). The higher renal NE spillover in the obese was not attributable to greater washout of the transmitter from an elevated renal blood flow, as mean renal plasma flows were similar in obese and lean men, 813 and 718 ml/min, respectively (difference not statistically significant). Whole body NE spillover rates were similar in the two groups. Although NE spillover from the heart tended to be decreased in obese subjects, it did not reach statistical significance (Table 1).

### Whole Body Leptin Secretion Rates

Renal clearance of leptin was  $135 \pm 26$  ml/min, equating to a removal rate of  $1,245 \pm 396$  ng/min, which was the estimated leptin secretion rate (Fig. 2).

### Whole Body Leptin Secretion in Obesity

As expected, arterial plasma leptin concentrations were significantly higher in obese than in lean men,  $10.2 \pm 1.2$  ng/ml vs.  $2.9 \pm 0.3$  ng/ml;  $P < 0.001$ . This was not attributable to any reduction in clearance of leptin by the kidneys, which was not different in the obese men (Fig. 3). The elevation in plasma leptin concentration in obese men was due to substantially greater whole body leptin release,  $1,950.8 \pm 643.5$  ng/min, compared with  $382.9 \pm 124.0$  ng/min in lean men;  $P = 0.044$  (Fig. 2).

### Linkage Between Leptin Secretion Rate and Sympathetic Nervous Activity

In lean and obese subjects combined, whole body leptin secretion was unrelated to adrenal medullary secretion of epinephrine or to cardiac NE spillover (Fig. 4). In contrast, a statistically significant direct relationship of low order was evident between leptin secretion rate and total NE spillover, the measure of overall sympathetic nervous system activity ( $r = 0.498$ ,  $P < 0.05$ ). The statistical significance of this relationship disappears with omission of the outlier with highest total NE spillover rate. A stronger relationship was present with renal NE spillover ( $r = 0.66$ ,  $P < 0.001$ ; Fig. 4).

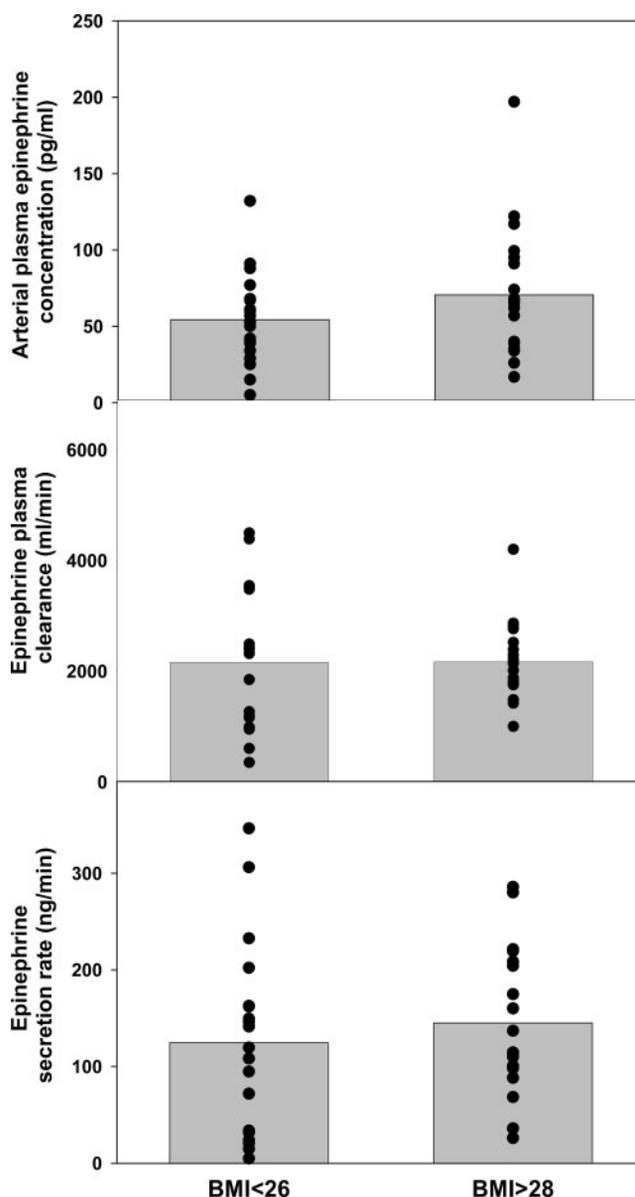


Fig. 1. Arterial plasma epinephrine concentration (top), epinephrine plasma clearance rate (middle), and epinephrine secretion rate (bottom) in lean and obese men. There were no significant differences between groups in epinephrine secretion rates.

### Regional Leptin Kinetics and Extra-Adipocyte Leptin Release to Plasma

There was no net input of leptin to plasma from the heart or hepatomesenteric circulation (Fig. 2; Table 2). A step-up in the plasma concentration of leptin was present across the brain circulation (Table 2). Unilateral jugular leptin spillover was  $264 \pm 87$  ng/min. Total brain leptin release to plasma was taken to be twice this value,  $529 \pm 175$  ng/min (46).

On the basis of the value for whole body leptin secretion,  $1,245 \pm 396$  ng/min, with subtraction of regional leptin fluxes across the brain, heart, and hepatomesenteric circulation, a maximum value of adipocyte-derived leptin release to plasma for all subjects combined was estimated to be  $716.4$  ng/min (Fig. 2). This figure relies on the assumption that inputs to

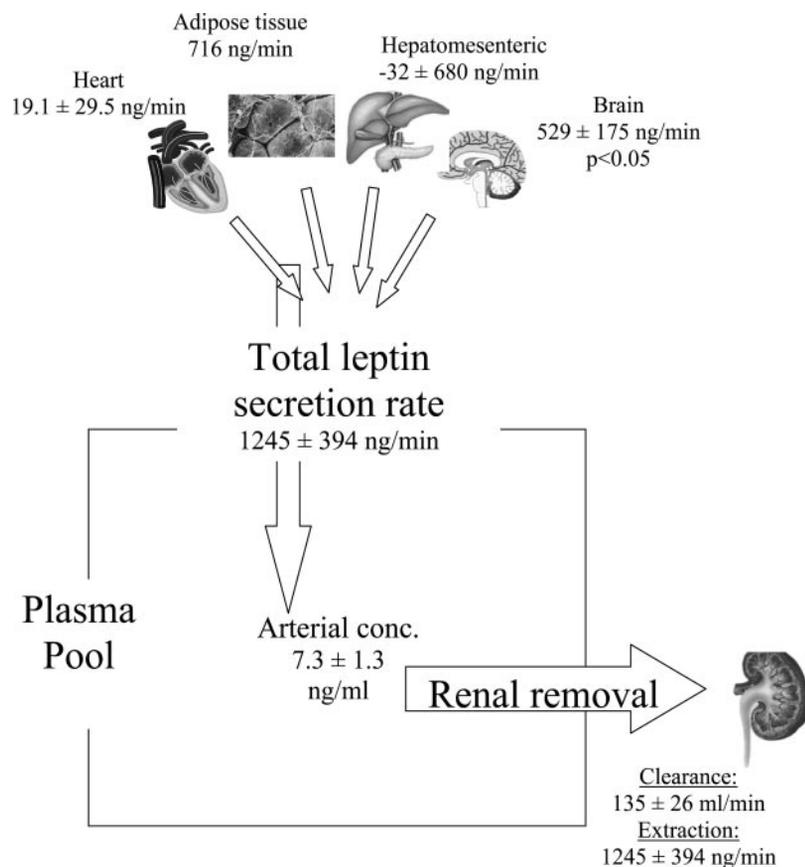


Fig. 2. Plasma leptin kinetics in men across a broad range of adiposity. The whole body leptin release rate to plasma was derived, at steady state, from renal extraction of leptin from plasma. Principal inputs of leptin to plasma were from adipose tissue and brain.

plasma from the lungs, skeletal muscle, and the urogenital system are small and can be discounted.

#### Brain Leptin Overflow in Obesity

The unilateral rate of overflow of leptin from the brain into an internal jugular vein was significantly greater in obese ( $467 \pm 160.4$  ng/min) than in lean men ( $80.0 \pm 29.3$ ;  $P = 0.045$ ; Fig. 5; Table 3).

#### Brain Serotonin Turnover and NPY Overflow in Obesity

The unilateral overflow of the principal serotonin metabolite, 5-HIAA, into the internal jugular vein was greater in obese than in lean men ( $227.8 \pm 112.1$  vs.  $21.6 \pm 14.5$  ng/min;  $P = 0.019$ ; Fig. 5). Although there was a trend for the correlation between serotonin turnover and total NE spillover rate, it did not reach statistical significance,  $r = 0.439$  ( $P = 0.06$ ). NPY overflow into the internal jugular vein was also greater in obese than in lean men:  $12.9 \pm 1.4$  vs.  $5.3 \pm 2.2$  ng/min ( $P = 0.042$ ; Fig. 5). Although leptin release from the brain and brain serotonin turnover were greater in obese men, there was no interindividual correlation between these two variables ( $r = 0.06$ ). No similar analysis was possible for brain NPY overflow measurements, which were made in the absence of leptin and serotonin turnover measurements, in a different patient subset.

#### DISCUSSION

We have investigated here regional leptin production in obese men and examined its relationship with NE turnover.

#### Reduced Catecholamine Thermogenesis Hypothesis

The sympathoadrenal system is an important contributor to the regulation of energy expenditure and, based primarily on results derived from experimental models of obesity (38), reduced sympathetic nervous activity and epinephrine secretion have been assumed to play a major role in the development of human obesity (3). Although some clinical studies have given support to the idea of reduced sympathoadrenal activity in the pathogenesis of obesity (33), recent evidence derived from sympathetic nerve recordings (clinical microneurography) (15) and from measurement of NE spillover (13) does not support the earlier claims. Although whole body NE spillover rates in obese humans with normal blood pressure are unremarkable, sympathetic neural outflow to the kidneys and to the skeletal muscle vasculature is increased (13, 15). Our findings here of unremarkable total NE spillover rates in obese men and increased NE spillover from the kidneys, accompanied by somewhat reduced cardiac NE spillover, conform well with the earlier observations (35).

Although the present results and other more recent evidence from studies in human obesity indicate that sympathetic nervous activity is actually increased in human obesity (13), the case is still open for the importance of reduced adrenal medullary secretion of epinephrine in obesity pathogenesis. Epinephrine does have important influences on metabolic rate (34), and suppressed adrenal medullary function has been proposed to be particularly associated with the development of central adiposity (40). Our finding of similar epinephrine

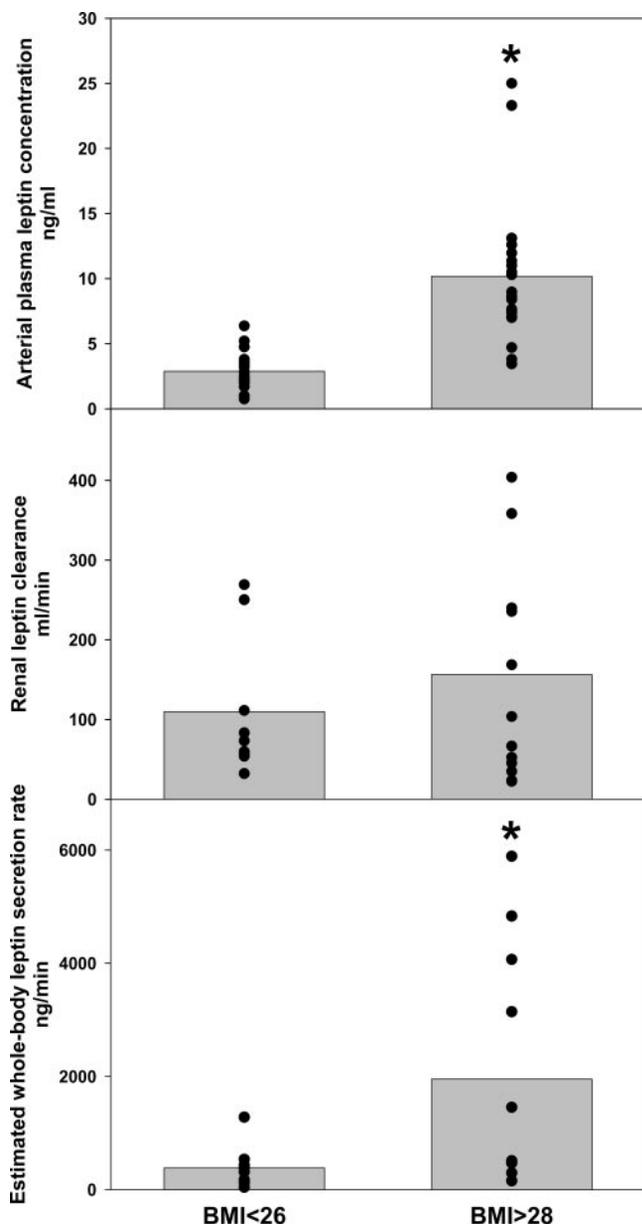


Fig. 3. Arterial plasma leptin concentration (*top*), renal leptin clearance (*middle*), and estimated whole body leptin secretion rate (*bottom*) in lean and obese men. \* $P < 0.05$ ; significant comparisons between groups.

secretion rates in obese and lean men argues against the hypothesis that low adrenal medullary secretion of epinephrine contributes to the development of obesity (24, 49).

#### Whole Body Leptin Secretion

Because clearance of leptin from plasma is primarily by renal removal (4), it was possible to estimate, for men at steady state during fasting, whole body leptin release to plasma from renal leptin excretion, derived from measurement of leptin extraction from plasma in transit through the kidneys by use of renal vein sampling. Perhaps not surprisingly, the estimated whole body leptin secretion rate was significantly elevated (5-fold increase) in the obese men. Mean renal clearance of leptin was similar in obese and lean men ( $156 \pm 41$  and  $110 \pm$

$29$  ml/min, respectively), making no contribution to the higher plasma leptin concentrations that characterize obesity.

#### Links Between Whole Body Leptin Secretion and Sympathetic Nervous Activity

Leptin and the sympathoadrenal system appear to be intimately linked (5). It has been suggested that there may be a two-way interaction between leptin and the sympathetic nervous system, perhaps constituting a regulatory feedback loop, with leptin acting within the hypothalamus to cause activation of central sympathetic outflow and stimulation of adrenal medullary release of epinephrine (9) and, conversely, with the sympathetic nervous system inhibiting leptin release from white adipose tissue (42). Increases in sympathetic outflow to the kidneys, adipose tissue, and the skeletal muscle vasculature and in the neural traffic to the adrenal gland are well documented after both intravenous and intracerebroventricular leptin administration in experimental animals (6, 8, 17). The effect of leptin infusions on sympathetic activity in humans, however, has not been definitively studied to this point, although daily subcutaneous injections of leptin were without effect (26). Given this uncertainty, we evaluated whether an association existed between regional sympathetic activity and whole body leptin secretion rate in the lean and obese men studied.

Whole body leptin secretion was elevated in the obese men. Of measures of sympathoadrenal function that we made, both total and renal NE spillover rates correlated directly with the whole body leptin secretion rate. Although these cross-sectional data perhaps suggest that hyperleptinemia may be the prime mover underlying the sympathetic nervous activation present in human obesity, given the experimental evidence from leptin administration in rodents (6, 8, 17), any causal link in humans is at best no more than suggestive, and more compelling and direct results would be needed to definitively establish leptin as a driving force in the renal sympathetic activation of human obesity and obesity-related hypertension. It should also be stated that the association we note here, of sympathetic activity with total plasma leptin levels, may not exist for free plasma leptin (39). Hyperinsulinemia (23) and obstructive sleep apnea (31), both common in obesity, have also been proposed as mechanisms of the sympathetic activation present. Obstructive sleep apnea was not obvious in these patients from interviews with them and their partners, but sleep laboratory monitoring was not performed. The possibility of age being a significant confounder in the present study by contributing to the sympathetic activation in the obese, given that they were on average 8 yr older than the lean men (a statistically nonsignificant difference), can be discounted. The influence of an age difference of  $<10$  yr on sympathetic activity is very much smaller than what we observe here (37).

#### Adipocyte and Extra-Adipocyte Release of Leptin

Evidence from studies in experimental animals and humans indicates that leptin, in addition to its release from adipocytes, is also released from multiple nonadipose sites (1, 14, 29), one of these being the brain (14, 29). This putative brain leptin system remains very incompletely categorized, and possible relationships to other brain neuronal systems regulating feeding behavior and energy expenditure are largely unexplored. We used simultaneous arteriovenous blood sampling to quan-

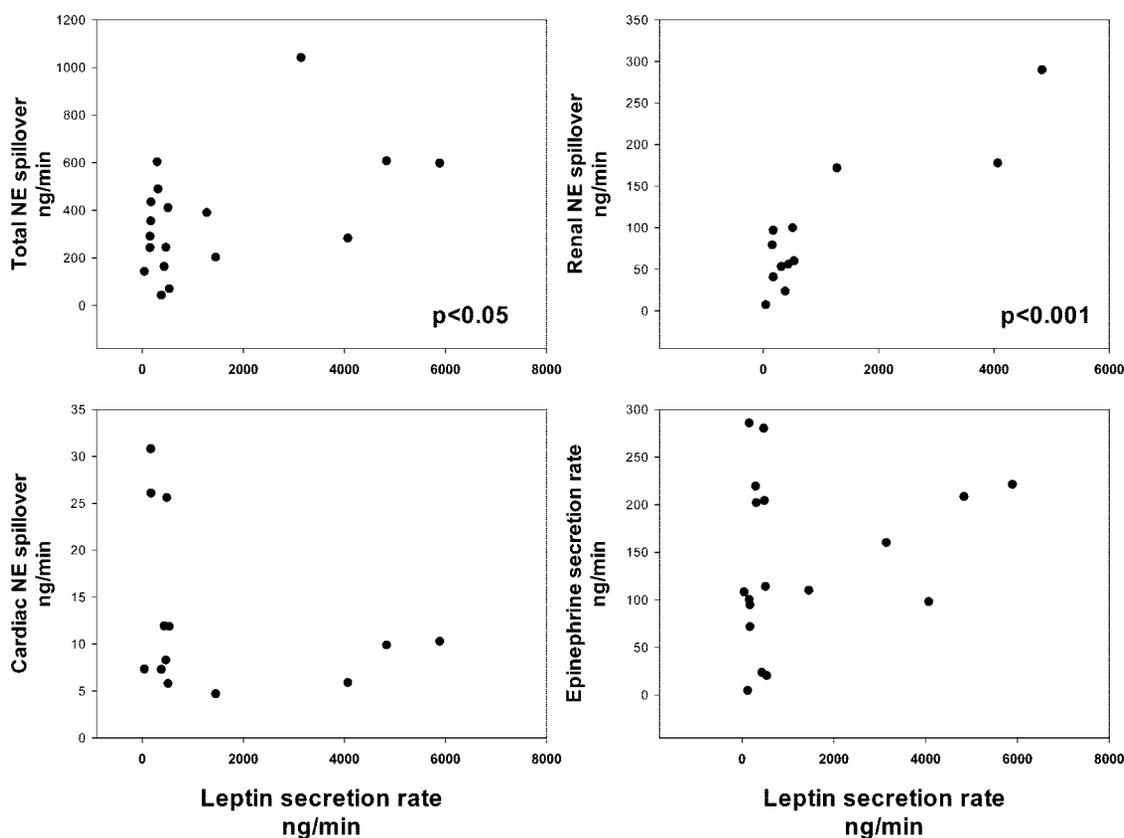


Fig. 4. Relation of whole body leptin secretion rate to measures of sympathetic nervous activity and to epinephrine secretion rates across a broad range of leptin values in men of widely differing adiposity. Estimated whole body leptin secretion rate correlated directly with total and renal norepinephrine (NE) spillover.

tify leptin release to plasma from individual organs, including internal jugular venous blood sampling to test for leptin efflux from the brain.

The concentration of leptin, on average, was higher in internal jugular venous than in arterial plasma, in line with our previous studies (14, 46), suggesting that leptin is released from the brain to plasma. Alternative explanations for the observed step-up in transcerebral plasma leptin concentration, such as contamination of jugular venous drainage by leptin from brain-associated adipocytes, are unlikely. Internal jugular

venous sampling was performed high up at the base of the brain, excluding the possibility of sampling derived from the venous drainage of the face. There is negligible adipose tissue associated with the brain, being confined largely to minor deposits of adipose bodies present in the cavernous sinus (44). The proportion of plasma leptin deriving from brain leptin release into the jugular veins was surprisingly large (>40%). Leptin mRNA and protein have been unequivocally demonstrated in the rat brain (29).

In contrast to our previous report (45), we found only trifling release of leptin from the heart. No basis for this different finding is apparent other than, perhaps, that the present study was confined to men. Similarly, there was no net release of leptin into the hepatomesenteric circulation, leptin concentrations being identical in arterial and hepatic venous plasma. Although this was unexpected, it being thought that there would be substantial leptin release from omental adipose tissue, the finding is in agreement with an earlier report based on central venous catheter sampling from the hepatic vein in humans (18). There are, however, two factors that might confound our observation of an absence of leptin overflow into the hepatic vein. First, it is possible that leptin released from omental adipose tissue might have been cleared from plasma during passage through the liver, although no direct confirmation of hepatic clearance of plasma leptin is available as yet. Hepatic clearance of leptin, if it exists, might materially reduce the contribution of the omentum to systemically circulating leptin. In addition, there is a second, technical caveat, namely

Table 2. Regional plasma leptin kinetics in men across a broad range of adiposity

Plasma Leptin Concentration, ng/ml	Regional Plasma Flow, ml/min	Regional Leptin Flux, ng/min
<b>Brain</b>		
ART $5.7 \pm 0.7$	$211.2 \pm 15.7$	$264.4 \pm 87.2$
IJV $7.0 \pm 0.9$		
<b>Kidney</b>		
ART $7.7 \pm 1.4$	$774.3 \pm 72.0$	$-1,245.3 \pm 393.5$
RV $6.1 \pm 1.0$		
<b>Heart</b>		
ART $6.7 \pm 1.1$	$86.7 \pm 4.8$	$19.1 \pm 29.5$
CS $6.9 \pm 1.0$		
<b>Hepatomesenteric</b>		
ART $8.9 \pm 1.9$	$847.7 \pm 95.0$	$-32.2 \pm 680.5$
HV $9.1 \pm 1.8$		

Values are means  $\pm$  SE. ART, arterial; IJV, internal jugular vein; RV, renal vein; CS, coronary sinus; HV, hepatic vein

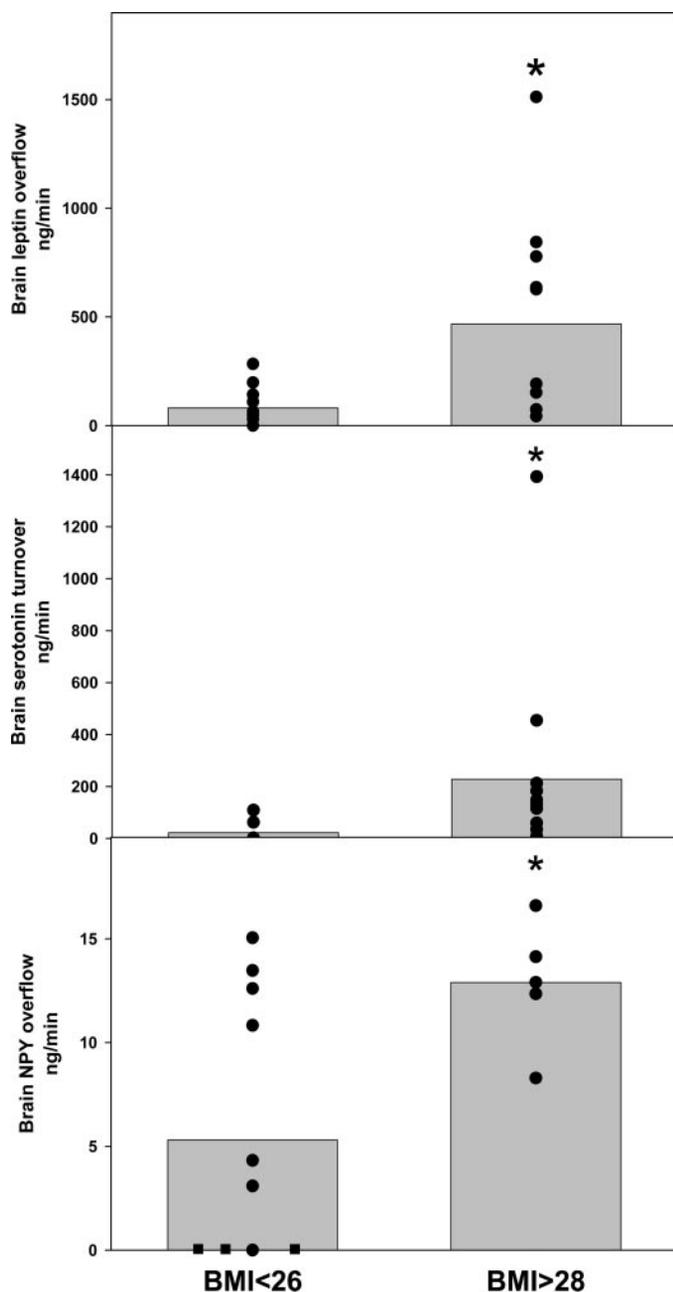


Fig. 5. Men with body mass index (BMI) greater than 28 had a significantly higher leptin (*top*), 5-hydroxyindoleacetic acid (5-HIAA, *middle*), and neuropeptide Y (NPY, *bottom*) unilateral overflow into an internal jugular vein than lean men. 5-HIAA overflow rate is indicative of brain serotonin turnover. \* $P < 0.05$ ; significant comparisons between groups.

that the high blood flow through the liver, by minimizing arteriovenous plasma leptin concentration differences, could cause measurement error.

The rate of release of leptin to plasma by adipocytes was estimated by subtracting measured regional leptin production rates, specifically those for the brain, heart, kidney, and hepatomesenteric circulation, from the whole body leptin secretion rate. In this derivation we discounted any leptin production by the lungs, skeletal muscle, and urogenital system. There appears to be no pulmonary leptin production (18), but assuming a negligible contribution by skeletal muscle and the urogenital

system is perhaps problematic. As anticipated, the derived leptin release rate for adipose tissue was markedly elevated in obesity. This increase in leptin production rate appears to be due both to an increase in fat cell number and to overexpression of leptin by adipocytes (19).

#### *Influence of Obesity on Release of Leptin from the Brain*

Leptin overflow from the human brain has previously been demonstrated to be influenced by gender, being greater in women (46). We here confirm an earlier preliminary finding (46) that an additional influence is adiposity, leptin release from the brain to plasma being markedly higher in obese than in lean men. The notion that leptin resistance might exist in human obesity because of a failure of leptin to enter the brain and access its sites of action in the hypothalamus would now seem to be unlikely, given direct experimental evidence of brain production of leptin (29) and our observation here in humans of leptin release from the brain.

#### *Increased Brain Serotonin Turnover in Obesity*

We quantified brain serotonin turnover from the rate of overflow of the major metabolite of serotonin, 5-HIAA, into the internal jugular veins by use of our previously established methods (21). Brain serotonergic mechanisms have been linked experimentally with the CNS actions of leptin (16, 48). Mutant mice lacking functional 5-hydroxytryptamine-2C receptors are overweight due to an abnormality in feeding behavior (41). The hypothalamus is highly innervated by serotonin-containing fibers, and the serotonergic system has been the target of a number of drugs aiming to regulate body weight (25). In humans, selective serotonin reuptake inhibitors, when used in patients with depressive illness and panic disorder, commonly cause substantial weight gain (27).

Table 3. Unilateral brain leptin overflow, brain serotonin turnover, and brain neuropeptide Y overflow in obese and lean men

Concentration	IJV PF, ml/min	Brain Overflow, ng/min
Plasma leptin, ng/ml		
Lean		
ART $3.2 \pm 0.4$	$211.7 \pm 25.1$	$80.0 \pm 29.3$
IJV $3.6 \pm 0.4$		
Obese		
ART $8.4 \pm 0.5$	$210.7 \pm 19.5$	$467.3 \pm 160.4^*$
IJV $10.7 \pm 0.8$		
Plasma 5-HIAA, ng/ml		
Lean		
ART $8.5 \pm 0.9$	$203.1 \pm 32.7$	$21.6 \pm 14.5$
IJV $8.2 \pm 0.9$		
Obese		
ART $6.6 \pm 0.9$	$214.4 \pm 115.1$	$227.8 \pm 112.1^*$
IJV $7.4 \pm 0.9$		
Plasma NPY, pg/ml		
Lean		
ART $262.5 \pm 32.2$	$275.4 \pm 31.3$	$5.3 \pm 2.2$
IJV $278.2 \pm 33.3$		
Obese		
ART $293.4 \pm 41.9$	$280.0 \pm 31.3$	$12.9 \pm 1.4^*$
IJV $341.4 \pm 40.4$		

PF, plasma flow; 5-HIAA, serotonin; NPY, neuropeptide Y. \* $P < 0.05$ ; significant differences between groups.

Using our jugular venous sampling method for quantifying whole brain serotonin turnover, we have previously been able to detect brain serotonergic neuronal systems responsive to light (21) and anxiogenic serotonergic neuronal pathways activated in patients with panic disorder (10). We here additionally confirm our earlier preliminary finding (22) of activation of serotonergic neuronal pools in obesity, presumably those involved in feeding and satiety responses.

#### *Release of NPY from the Brain in Obesity*

NPY concentration is increased in the hypothalamus in some experimental genetic models of obesity (2), and neuronal NPY in the arcuate nucleus has been implicated in the effects of leptin on feeding behavior. Previously we have reported that there is no overflow of NPY from the brain to plasma in lean men (30), and we confirm this in the present study. In contrast, brain release of NPY to plasma was evident in obese men.

These results indicate that leptin is released within the brain, and at an increased rate in obese humans, in whom activation of brain serotonergic and NPY mechanisms also exists. Although leptin release from the brain and brain serotonin turnover were greater in obese men, there was a nonsignificant trend only for interindividual correlation between these two variables, which argues against a close causal link. No similar inference concerning the presence or absence of a causal relation of brain NPY release to brain leptin release and serotonin turnover is possible, as NPY was measured without accompanying leptin and serotonin measurements in a separate patient subset.

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