

SHORT COMMUNICATION

Obesity, leptin resistance, and the effects of insulin reduction

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Leptin resistance is a hallmark of obesity, but its etiology is unknown, and its clinical measurement is elusive. Leptin-sensitive subjects have normal resting energy expenditure (REE) at a low leptin concentration, while leptin-resistant subjects have a normal REE at a higher leptin concentration; thus, the ratio of REE:Leptin may provide a surrogate index of leptin sensitivity. We examined changes in REE and leptin in a cohort of 17 obese subjects during experimental weight loss therapy with the insulin-suppressive agent octreotide-LAR, 40 mg i.m. q28d for 6 months. Six subjects lost significant weight (> 10%) and BMI (> -3 kg/m²) with a 34% decline in leptin and a 46% decrease in insulin area under the curve (IAUC) to oral glucose tolerance testing. These subjects maintained their pretreatment REE, and thus exhibited a rise in REE:Leptin, while the other 11 showed minimal changes in each of these parameters. For the entire cohort, the change in IAUC correlated negatively with the change in REE:Leptin. These results suggest that the REE:Leptin ratio, while derivative, may serve as a useful clinical indicator of changes in leptin sensitivity within obese subjects. They also support the possibilities that hyperinsulinemia may be a proximate cause of leptin resistance, and that reduction of insulinemia may promote weight loss by improving leptin sensitivity.

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Introduction

Obese subjects are almost uniformly hyperleptinemic, but the hypothalamus is unable to transduce this leptin signal to reduce body weight, termed 'leptin resistance'. Leptin resistance also prevents exogenous leptin administration from promoting weight loss.¹ The response to most weight loss regimens plateaus rapidly due to the rapid down-regulation of peripheral leptin levels below a 'leptin threshold'. Leptin decline causes the ventromedial hypothalamus (VMH) to sense a reduction in peripheral energy stores, which modulates a decrease in resting energy expenditure (REE) to conserve energy, analogous to that seen in starvation.² However, Rosenbaum *et al*³ have shown that once weight is reduced through marked food restriction, exogenous administration of leptin is then able to increase REE back to baseline and permit further weight loss,

suggesting that some factor or process associated with weight loss improves leptin sensitivity.

Obese subjects also are almost uniformly hyperinsulinemic.⁴ Insulin stimulates leptin production from adipocytes via an indirect mechanism dependent on lipogenesis.^{5–7} Most weight loss regimens (through caloric restriction) cause lipolysis, which leads to acute leptin suppression, but reduces hyperinsulinemia more slowly.⁸ We postulated that: (1) hyperinsulinemia impairs hypothalamic leptin sensitivity; and that (2) reduction of insulinemia improves leptin sensitivity and promotes weight loss. We chose to suppress insulin directly at the β -cell using the somatostatin agonist octreotide-LAR (Novartis; East Hanover, NJ, USA),⁹ as this drug has previously been shown to suppress insulin secretion and safely promote weight loss without causing diabetes mellitus, both in children with obesity due to hypothalamic insult^{10,11} and in select adults whose obesity is secondary to insulin hypersecretion.^{12,13} Other insulin sensitizers, such as thiazolidinediones, while lowering plasma insulin levels, increase energy deposition into adipose tissue.¹⁴

Measurement of leptin sensitivity in humans has been elusive, as there is no static biochemical measure of leptin's

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effect. Lean, leptin-sensitive individuals are able to maintain a high REE at a low leptin level, whereas high leptin levels are required to maintain a normal REE in obese, leptin-resistant subjects. Furthermore, if an obese subject attempted to lose weight, their leptin level would fall, triggering the hypothalamic response to starvation and decreasing REE. We therefore hypothesized that changes in the REE:Leptin ratio would reflect changes in leptin sensitivity.

We evaluated changes in the REE:Leptin ratio in 17 obese adults who underwent experimental weight loss therapy with octreotide-LAR and correlated the change in REE:Leptin to the change in insulin.

Subjects and methods

This study was approved by the University of Tennessee Institutional Review Board and the Scientific Advisory Committee of the University of Tennessee General Clinical Research Center (UT GCRC), and all subjects gave informed consent prior to participation. A total of 17 obese subjects underwent 6 months of experimental weight loss therapy using octreotide-LAR, 40 mg i.m. every month.¹²

Subjects were admitted to the UT GCRC at the beginning and end of this 6-month trial. On arrival in the morning, each subject underwent REE using indirect calorimetry using a Medical Graphics CPX metabolic cart (St Paul, MN, USA). With a new drying tube in place each day, the O₂ and CO₂ analyzers were calibrated per protocol (a combined internal and manual adjustment system) based on the ambient temperature and barometric pressure. In addition, the breathing capacity analyzer was calibrated per protocol with a three-calibration syringe using multiple measures. Measurements were taken in a resting and fasting state for 30 min using a mouthpiece containing a non-rebreathing valve with obstruction of nasal breathing with a nose clamp. REE was derived from the respiratory exchange ratio and the respiratory quotient. After the REE was completed, an intravenous line was started, serum leptin was obtained, and thereafter each subject underwent 3-h oral glucose tolerance testing (OGTT).^{12,15} The insulin area under the curve (IAUC) is an index of the magnitude of the insulinemia and was computed from the insulin excursion.¹⁶

Subjects were also analyzed for total tissue, fat mass, and lean mass at weeks 0 and 24 by DEXA, using a Lunar DPX-L machine (Madison, WI, USA). Subjects received 0.06 mrem of radiation during the 40 min scan. Autowidth and length settings were utilized to reduce scan time and radiation exposure. The appropriate energy level was determined individually based on each subject's body habitus. The week 24 scan was analyzed by comparison of regions of interest to the reference (week 0) scan. We were able to obtain accurate data in only 12 subjects, due to the 137 kg upper weight limit of the DEXA table.

Chemical analysis

Serum glucose during OGTT was measured by the glucose oxidase method.¹⁷ Serum immunoreactive insulin (μ U/ml) levels from each OGTT were measured by standard double-antibody radioimmunoassay (RIA) (Linco Research; St Louis, MO, USA). IGF-1 and leptin was measured by double-antibody RIA (Endocrine Sciences; Calabasas Hills, CA, USA). All other laboratory studies were performed by Memphis Pathology Laboratory (Memphis, TN, USA).

Statistical analysis

All statistical analysis was performed using the R programming language (www.r-project.org). As the distribution of IAUC was skewed, these values were log₂-transformed to make their distribution amenable to statistical analysis. Association between key variables and change in BMI was measured by Pearson's product moment correlation coefficient. The *P*-values and correlation coefficients were calculated using Fisher's Z-transform. As DEXA data were not obtainable in a proportion of the subjects, we could not evaluate the change in the REE:Leptin ratio as a function of fat-free mass.² Instead, we analyzed the association by linear regression analysis of the change in the REE:Leptin ratio and the change in IAUC, adjusting by mean BMI (average of baseline and final BMI).

Results

Baseline demographics, energy expenditure, and biochemical analysis of this population are listed in Table 1. Six subjects lost BMI greater than 3 kg/m² (high responders), seven lost between 0 and 3 kg/m² (low responders), and five subjects gained BMI (nonresponders).

In comparison to the low and nonresponders, the group of six high responders exhibited decline in weight, BMI, leptin, and IAUC with octreotide-LAR therapy, but without significant change in REE.

Individually, the changes in leptin and in REE were not associated with change in BMI (Table 2). However, changes in the REE:Leptin ratio and in IAUC were associated with change in BMI (*P* = 0.008 and 0.006, respectively).

Linear correlation analysis between Δ IAUC and Δ REE:Leptin after insulin suppression with octreotide-LAR is depicted in Figure 1. Although no correlation between Δ IAUC vs either Δ leptin or Δ REE could be discerned, a significant negative relationship between Δ IAUC and the Δ REE:Leptin was noted (*P* = 0.039).

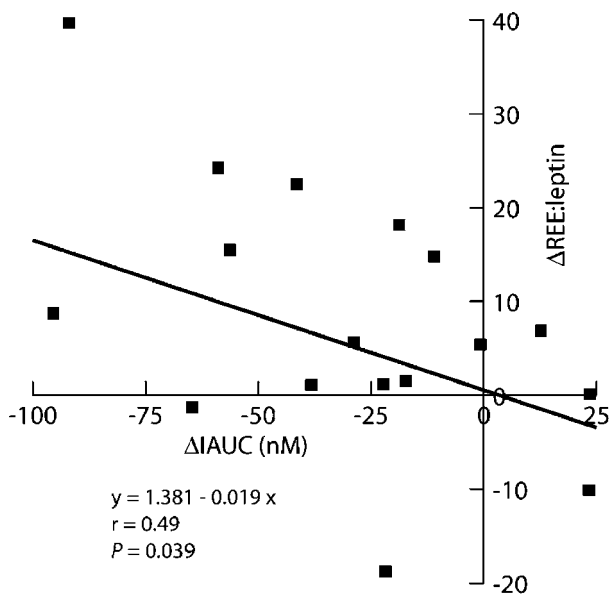
The change in the REE:Leptin ratio was associated with changes in IAUC after adjusting for BMI (Table 3) (*P* = 0.077). A two-fold increase in IAUC is associated with a decline in the REE:Leptin ratio of 8.7. Given the baseline REE:Leptin ratio of 31.1, this is a very large effect, although not statistically significant due to the small sample size.

Table 1 Clinical and biochemical characteristics of the cohort and change during study (mean \pm s.d.)

Variable	n	Mean \pm s.d.
Age (y)	17	35.9 \pm 9.9
Waist:hip ratio	17	0.82 \pm 0.06
Weight, month 0	17	125.0 \pm 28.4
Δ Weight	17	-4.4 \pm 6.8
BMI (kg/m ²), month 0	17	45.1 \pm 6.5
Δ BMI (kg/m ²)	17	-1.5 \pm 2.5
Leptin, month 0 (ng/ml)	17	64.8 \pm 24.0
Δ Leptin	17	-14.3 \pm 16.6
IGF-1, month 0 (ng/ml)	17	142.9 \pm 44.0
Δ IGF-1	17	-30.4 \pm 32.4
Fat-free mass (DEXA; kg)	12	52.0 \pm 4.7
Δ Fat-free mass	12	-2.5 \pm 3.6
IAUC, Month 0 (nM)	17	17003 \pm 13470
Δ IAUC	17	-4167 \pm 4954
REE, Month 0 (kcal/day)	17	1814 \pm 454
Δ REE	17	-88 \pm 238
REE:Leptin, Month 0	17	31.1 \pm 11.2
Δ REE:Leptin	17	8.0 \pm 13.8

Table 2 Association between change in BMI and changes in key variables

Variable	Correlation	95% confidence intervals	P-value
Δ leptin	0.318	-0.192-0.692	0.214
Δ IAUC	0.619	0.197-0.847	0.008*
Δ REE	-0.159	-0.594-0.348	0.542
Δ REE:Leptin	-0.635	-0.855 to -0.223	0.006*

* $P < 0.05$.**Figure 1** Correlation between the change in insulin area under the curve (Δ IAUC), as a measure of the change in insulinemia, and the change in the resting energy expenditure:leptin ratio (Δ REE:Leptin), as an index of the change in leptin sensitivity, in response to 6 months of octreotide-LAR therapy for obesity.**Table 3** Regression analysis of change in REE:Leptin vs change in log IAUC and change in mean BMI

Variable	Estimate	s.e.	t-value	P-value
Intercept	-44.298	21.269	-2.083	0.0561
Δ log IAUC	-8.684	4.557	-1.906	0.0774
Mean BMI (kg/m ²)	1.07	0.461	2.321	0.0359*

* $P < 0.05$.

Discussion

Hyperinsulinemia and leptin resistance coexist in the overwhelming majority of obese subjects. Leptin resistance prevents normal leptin signal transduction at the VMH, permitting continued caloric intake and obesity. The cause of leptin resistance remains unknown. Several authors have examined the plasma:CSF leptin ratio and the role of the saturable leptin transporter, and have suggested that changes in leptin penetration at the blood-brain barrier may be one of the etiologies of leptin resistance.¹⁸⁻²⁰ Others have suggested that upregulation of intraneuronal SOCS-3 may contribute to the phenomenon of leptin resistance.²¹ In addition, in a high-fat-diet-induced obesity mouse model, El-Haschimi *et al*²² demonstrated that the STAT3 response to intracerebroventricular leptin administration was attenuated, suggesting a defect in neuronal leptin signal transduction.

One obstacle to successful human obesity therapy is the attainment of a negative plateau with most weight loss regimens. This plateau is associated with decline in serum leptin concentration and reduction of REE,²³ which is likely to impact negatively on the subject, promoting subjective fatigue and starvation, and thus reducing compliance. Improved leptin sensitivity would necessarily promote decreased caloric intake and body weight, with maintenance of REE at lower serum leptin concentration, and presumably obviate the obese state.

To examine this phenomenon, a clinical index of leptin sensitivity is required. Increases of leptin to maintain REE at a pre-morbid state would indicate leptin resistance, while maintenance of REE at a lower leptin level would suggest improved leptin sensitivity. Thus, we evaluated the utility of the REE:Leptin ratio as a surrogate measure for quantitating changes in leptin sensitivity within individuals. We interpret the association of the change in the REE:Leptin ratio with the change in BMI (Table 2) as an indication of improvement in leptin sensitivity with our experimental weight loss therapy. Leibel *et al*² have shown that weight reduction decreases REE when corrected for fat-free body mass. Unfortunately, our measure of body composition (DEXA) excluded more than one-third of our subject population from this analysis due to the weight limitation of the DEXA table, and thus we were unable to assess the role of changes in the lean body mass in the validation of the REE:Leptin ratio. In addition, the utility of the REE:Leptin ratio to assess leptin resistance cross-sectionally in a diverse group of obese individuals has not yet been established.

Guyen *et al*²⁴ demonstrated that both leptin and insulin levels remained elevated in obese subjects after weight reduction relative to BMI-matched never-obese controls, consistent with persistent leptin resistance. Conversely, Rosenbaum *et al*³ demonstrated that forced weight loss led to reductions in leptin and REE; but in this state, these subjects became more responsive to exogenous leptin, and improved REE back to baseline in order to feel better, and possibly lose more weight, inferring that the weight reduced state resulted in improvement in leptin sensitivity. Indeed, other pharmacotherapeutic weight loss strategies in rats appear to be adjunctive in increasing leptin sensitivity.²⁵ We achieved the same effect in the current study, that is, weight loss, reduced leptin, and maintenance of REE, by instead suppressing insulin secretion with octreotide-LAR. The similarity between these two weight loss paradigms is the reduction of insulinemia, either through caloric restriction or through pharmacologic means. Indeed, other maneuvers that reduce insulinemia, such as the low-carbohydrate diet, also appear to be efficacious in promoting weight loss while not causing fatigue and lassitude.²⁶

The change in IAUC did not correlate with changes in either REE or leptin *per se* (data not shown), but it did correlate negatively with the change in the REE:Leptin ratio, adjusting for mean BMI. Although a causal relationship is not established, our data suggest that hyperinsulinemia is associated with leptin resistance, and that reduction in insulinemia improves leptin sensitivity. Discordant short-term reductions in leptin vs insulin may account for the difficulty in achieving weight loss with standard therapies and the reason for rebound weight gain once treatment is discontinued. Insulin and leptin act through different receptors on the same VMH neurons to activate the same second messenger system (insulin receptor substrate 2 (IRS2)/phosphatidyl inositol-3-kinase (PI3K)),²⁷ which, in turn, mediates the anorexigenic effects of both of these two hormones. In addition, IRS2 recruitment appears to be necessary for leptin's transduction of the JAK2-STAT3 pathway.²⁸

This concept of the REE:Leptin ratio is derivative and currently does not have external validation. However, no other clinical measures of leptin sensitivity have yet been advanced, short of leptin administration, which is currently not feasible or practical. However, measurement of the REE:Leptin ratio is easily performed and can be examined in other weight loss studies. Whether hyperinsulinemia is a proximate cause of leptin resistance and whether the hypothalamic IRS2/PI3K second messenger system is dysfunctional remains to be determined. However, this study highlights the promise of reduction of insulinemia as a primary goal in successful weight loss therapy.

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