Leptin production during early starvation in lean and obese women

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Departments of 1Internal Medicine and 3Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110 and 3University College London Medical School, Whittington Hospital, London N2 9QA, United Kingdom

Klein, Samuel, Jeffrey F. Horowitz, Michael Landt, Stephen J. Goodrick, Vidya Mohamed-Ali, and Simon W. Coppack. Leptin production during early starvation in lean and obese women. Am. J. Physiol. Endocrinol. Metab. 278: E280–E284, 2000.—We evaluated abdominal adipose tissue leptin production during short-term fasting in nine lean [body mass index (BMI) 21 ± 1 kg/m2] and nine upper body obese (BMI 36 ± 1 kg/m2) women. Leptin kinetics were determined by arteriovenous balance across abdominal subcutaneous adipose tissue at 14 and 22 h of fasting. At 14 h of fasting, net leptin release from abdominal adipose tissue in obese subjects (10.9 ± 1.9 ng·100 g tissue·h−1) was not significantly greater than the values observed in the lean group (7.6 ± 2.1 ng·100 g tissue·h−1). Estimated whole body leptin production was approximately fivefold greater in obese (6.97 ± 1.18 µg/min) than lean subjects (1.25 ± 0.28 µg/min) (P < 0.005). At 22 h of fasting, leptin production rates decreased in both lean and obese groups (to 3.10 ± 1.31 and 10.5 ± 2.3 ng·100 g tissue·h−1, respectively). However, the relative declines in both arterial leptin concentration and local leptin production in obese women (arterial concentration 13.8 ± 4.4%, local production 10.0 ± 12.3%) were less (P < 0.05 for both) than the relative decline in lean women (arterial concentration 39.0 ± 5.5%, local production 56.9 ± 13.0%). This study demonstrates that decreased leptin production accounts for the decline in plasma leptin concentration observed after fasting. However, compared with lean women, the fasting-induced decline in leptin production is blunted in women with upper body obesity. Differences in leptin production during fasting may be responsible for differences in the neuroendocrine response to fasting previously observed in lean and obese women.

adipose tissue; lipid metabolism; obesity

THE DISCOVERY OF THE OB GENE and its protein product, leptin, has furthered our understanding of the regulation of energy balance. Leptin is produced primarily by adipose tissue and is secreted into the bloodstream where it is delivered to the brain and ultimately to the hypothalamus (8). Specific receptors for leptin have been characterized in hypothalamic nuclei that express neuropeptides and neurotransmitters involved in the regulation of food intake and metabolic rate (32).

Therefore, leptin provides a unique feedback signaling system that transmits information regarding adipose tissue energy stores to the central nervous system. Disruption of this system by either impaired leptin production or leptin receptor function causes excessive food intake, decreased energy expenditure, and severe obesity (17, 35).

Although considerable attention has been focused on the importance of leptin in the pathogenesis of overfeeding, leptin's dominant function may be its involvement in the neuroendocrine response to underfeeding. Plasma leptin concentration decreases markedly within the first 24 h of fasting (4, 14, 24). The decline in circulating leptin during early fasting is much greater than the change in fat mass; therefore, the fasting-induced decrease in leptin cannot be attributed solely to changes in body composition. In addition, the relative decrease in plasma leptin concentration is much greater than the relative decrease in adipose tissue leptin gene expression (14), suggesting that alterations in both leptin production and clearance might contribute to the fall in plasma leptin levels. The decline in leptin during fasting may be responsible for suppressing sympathetic nervous system (SNS) activity and reproductive function and stimulating the secretion of steroid hormones (29). Preventing the decline in plasma leptin by exogenous leptin administration blunts the normal alterations that occur in gonadal, adrenal, and thyroid axes (1) and in energy expenditure (30).

We have recently found that many of the metabolic alterations that occur within the first 24 h of fasting, such as the decline in circulating insulin, the decrease in SNS activity, and the increase in lipolysis of adipose tissue triglycerides, are blunted in obese compared with lean persons (10); however, it is not known whether leptin metabolism during early fasting is also altered by obesity. Boden et al. (4) compared plasma leptin concentrations during short-term fasting in lean with obese subjects. In their study, the relative decline in plasma leptin concentration at the end of 52 h of fasting was similar in both groups, but a statistical analysis of the potential differences in plasma leptin response during the first 24 h of fasting was not performed.

The purpose of the present study was 1) to assess the relative importance of adipose tissue leptin production and plasma leptin clearance in regulating plasma leptin concentrations during fasting and 2) to evaluate...
whether adiposity influences the effect of short-term fasting on in vivo leptin metabolism. Net abdominal subcutaneous adipose tissue leptin production and an estimate of whole body plasma leptin clearance were determined at 14 and 22 h of fasting in lean and obese women. We hypothesized that 1) the rate of leptin production is the primary regulator of plasma leptin concentrations during fasting, and 2) obesity blunts the normal decline in plasma leptin concentration during early fasting.

METHODS

Subjects. Eighteen (9 lean and 9 obese) women participated in this study (Table 1), which was approved by the Institutional Review Board and the General Clinical Research Center of Washington University School of Medicine. All lean women had a body mass index (BMI) <25.0 kg/m² (range 19.7–24.5 kg/m²). All obese women had a BMI >35.0 kg/m² (range 35.9–44.3 kg/m²) and were considered to have upper body obesity (UBO), defined as a waist-to-hip circumference ratio >0.85 and waist circumference >100 cm. Fat mass and fat-free mass were determined by dual-energy X-ray absorptiometry (Hologic QDR 1000/W, Waltham, MA). Obese women had more than three times as much body fat mass, but a similar amount of fat-free mass, as the lean women (Table 1). All subjects completed a comprehensive medical examination, had normal fasting blood glucose concentration and lipid (cholesterol and triglyceride) profiles, and had no evidence of disease except for the presence of obesity.

Experimental procedure. Subjects were admitted to the General Clinical Research Center at Washington University School of Medicine in the evening before the study. At 1800 subjects consumed a standard meal containing 12 kcal/kg body wt for lean subjects and 12 kcal/kg adjusted body wt for obese subjects [adjusted body wt = ideal body wt + (actual body wt – ideal body wt) × (0.25)]. The energy content of the meal consisted of 55% carbohydrate, 30% fat, and 15% protein. At 2000, a liquid formula snack containing 40 g carbohydrate, 6.1 g fat, and 8.8 g protein (Ensure, Ross Laboratories, Columbus, OH) was ingested by all subjects. The next morning, after subjects had fasted overnight, a 20-gauge catheter was inserted into a radial artery for arterial blood sampling, and a 22-gauge catheter (Hydrocath, Viggo-Spectramed, Oxnard, CA) was placed in an abdominal vein draining abdominal subcutaneous adipose tissue (7). Four consecutive blood samples were obtained from the artery and abdominal vein simultaneously every 5 min from 0945 to 1000 and from 1745 to 1800 so that the last samples were taken at precisely 14 and 22 h of fasting, respectively.

Subcutaneous abdominal adipose tissue blood flow (ATBF) was measured by the 133Xe clearance technique (16). The standard deviation for repeated measurements using this technique in a group of subjects with a mean ATBF rate of 3.65 ml·100 g adipose tissue⁻¹·min⁻¹ was 0.9 ml·100 g adipose tissue⁻¹·min⁻¹ (31). Approximately 100 µCi of 133Xe dissolved in 0.15 ml of saline was slowly injected into abdominal subcutaneous adipose tissue, 3 cm from the umbilicus. A cesium iodide detector (Oakfield Instruments LTD, Eynsham, UK) was taped to the skin directly over the site of injection. The 133Xe clearance rate was measured by collecting 10-s counts (27) continuously for 15 min from 0945 to 1000 and from 1745 to 1800 (at 14 and 22 h of fasting, respectively).

Analytical procedures. Plasma leptin concentration was measured by RIA, as described previously (23).

Calculations. Subcutaneous ATBF was calculated from 133Xe clearance (16). The adipose tissue-to-blood partition coefficient for xenon was assumed to be 10 ml/g for lean and obese women (2). Subcutaneous adipose tissue plasma flow (ATPF) was calculated as ATBF (1 – hematocrit).

Net regional release of leptin from subcutaneous abdominal adipose tissue into plasma was quantified by calculating arteriovenous balance:

\[
\text{net regional release} = \frac{[\text{leptin}_1] - [\text{leptin}_0]}{\text{ATPF}}
\]

where [leptin₁] and [leptin₀] are leptin arterial and abdominal venous plasma concentrations, respectively. Whole body leptin production rate was estimated 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 micrograms per minute by [leptin₀] in micrograms per liter.

Statistical analysis. An ANOVA with repeated measures was used to test the significance of differences between lean and obese groups and the effect of fasting within each group. Significant F ratios from ANOVA were followed by the appropriate paired comparisons using the Neuman-Keuls procedure. A value of P ≤ 0.05 was considered to be statistically significant. All data are expressed as means ± SE.

RESULTS

Plasma leptin concentrations. At 14 h of fasting, arterial plasma leptin concentration was fivefold greater in obese than in lean subjects (P < 0.001) (Table 2). By 22 h of fasting, arterial plasma leptin concentration had declined significantly (P < 0.05) in both groups, but the percentage of decline was less in obese than in lean women (13.8 ± 4.4 and 39.0 ± 5.5%, respectively; P < 0.005) (Fig. 1). Abdominal vein plasma leptin concentrations were always greater than paired arterial concentrations in all subjects, indicating net leptin release from subcutaneous abdominal adipose tissue at both 14 h and 22 h of fasting (Table 2).

Leptin kinetics. At 14 h of fasting, net adipose tissue local leptin production tended to be greater in obese (10.94 ± 1.97 ng·100 g⁻¹·min⁻¹) than in lean (7.57 ± 2.14 ng·100 g⁻¹·min⁻¹) women, but the difference

### Table 1. Study subject characteristics

<table>
<thead>
<tr>
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<th>Lean Subjects</th>
<th>Obese Subjects</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>34.1 ± 5.2</td>
<td>35.8 ± 2.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.1 ± 0.8</td>
<td>37.1 ± 2.9</td>
</tr>
<tr>
<td>Body fat mass, %</td>
<td>27.0 ± 2.4</td>
<td>53.4 ± 1.6</td>
</tr>
<tr>
<td>Body fat mass, kg</td>
<td>14.5 ± 1.7</td>
<td>53.0 ± 6.1</td>
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<tr>
<td>Fat free mass, kg</td>
<td>41.1 ± 2.0</td>
<td>46.2 ± 2.5</td>
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Values are means ± SE.
LEPTIN PRODUCTION DURING FASTING

DISCUSSION

Circulating leptin represents an important communication pathway between adipose tissue and the brain during fasting. A decrease in plasma leptin concentration is part of the neuroendocrine response to fasting, which is critical for initiating the changes in substrate metabolism and energy expenditure necessary for survival. The results of the present study demonstrate that downregulation of adipose tissue leptin production occurs early in starvation and is responsible for the observed decrease in plasma leptin concentration. Moreover, we found that regulation of leptin production during fasting was altered by obesity; the declines in leptin production and plasma leptin concentration in our obese subjects were blunted compared with the decline in our lean group. These data support the notion that leptin is a component of the initial neuroendocrine response to fasting, and alterations in leptin metabolism may be responsible for some of the differences in the adaptation to fasting observed between lean and obese persons (10).

The mechanism responsible for the altered decline in adipose tissue leptin production during fasting in our obese women may be related to the effect of obesity on glucose metabolism. The results from a series of studies provide evidence that alterations in glucose metabolism may be responsible for initiating the early metabolic response to fasting, including changes in leptin production (4, 11, 13, 20). First, carbohydrate restriction, not energy restriction, initiates the changes in circulating hormones and substrate metabolism that occur during short-term fasting; providing daily energy requirements solely from lipid does not prevent the normal decrease in plasma insulin concentration, decrease in glucose production, or increase in lipolysis (13). Second, maintaining euglycemia by continuous low-dose glucose infusion (providing an average of ~90 g of glucose/day) during 72 h of fasting prevents the normal decline in plasma insulin and leptin concentrations (4). Third, the decrease in plasma leptin that occurs during hypocaloric feeding is directly correlated with the decrease in carbohydrate intake, but not with fat or protein intake (11). Fourth, glucose uptake and metabolism regulate leptin secretion by isolated adipocytes (20). We have recently found that the decrease in glucose production rate, plasma glucose concentration, and presumably glucose utilization that occurs during the first 24 h of fasting is blunted in obese compared with lean subjects (unpublished observations). There-

![Fig. 1. Values are means ± SE. Relative change in plasma leptin concentration and adipose tissue leptin production during short-term fasting (between 14 and 22 h of fasting) in lean and obese women. Data represent values obtained at 22 h of fasting, expressed as a percentage of 14-h fasting values. * Value significantly different from corresponding lean value, P < 0.05.](http://ajpendo.physiology.org/)

![Fig. 2. Values are means ± SE. Adipose tissue leptin production rates in lean and obese women at 14 and 22 h of fasting. * Value significantly different from 14-h-fast value, P < 0.05. + Value significantly different from corresponding lean value, P < 0.05.](http://ajpendo.physiology.org/)
fore, the results of the present study, in conjunction with data from previous investigations, suggest that a decrease in glucose utilization caused by carbohydrate restriction decreases both insulin secretion and adipose tissue leptin production. The subsequent decline in plasma insulin and leptin concentration represents key signals for initiating the metabolic response to fasting. Alterations in glucose metabolism associated with obesity may be responsible for the differences we observed in leptin production between our lean and obese subjects during early fasting.

An important component of the adaptive response to fasting is a decrease in SNS activity, which serves to conserve energy and thereby enhance survival. Adiposity affects the SNS response to fasting; norepinephrine spillover into plasma, an index of whole body SNS activity, decreases during short-term fasting in lean but not in obese subjects (6). Alterations in leptin metabolism may help explain these differences in whole body SNS activity between lean and obese persons during fasting because leptin is an important regulator of the SNS. Leptin administration has been shown to stimulate SNS activity in brown adipose tissue, kidney, adrenal gland, and hindlimb (5, 9). Therefore, it is possible that alterations in leptin production during fasting contribute to the differences in whole body SNS activity observed between lean and obese subjects. In addition, increased SNS activity decreases adipose tissue ob gene expression and plasma leptin concentration (33), providing a negative feedback signaling system between leptin production and SNS activation.

In the present study, we evaluated leptin production during early fasting in lean women and women with UBO: therefore, we cannot be certain that our results are representative of obese men or of women with lower body obesity. Although several studies suggest that gender has an independent effect on basal plasma leptin concentrations (12, 22, 26), the relationship between body fat distribution and plasma leptin concentration is unclear because of conflicting results from different studies (3, 21, 36). We are not aware of any studies that evaluated whether fat distribution or gender influences fasting-induced changes in either leptin production or plasma leptin concentration.

A potential limitation of our study is that leptin production was measured in only one fat depot (subcutaneous abdominal fat), which may not represent whole body leptin metabolism; however, it is unlikely that our conclusions would change had we obtained data from additional adipose tissue sites because of the major contribution of subcutaneous fat to whole body leptin production. Subcutaneous fat accounts for >90% of total body fat in both lean and obese women (15, 25). Moreover, leptin mRNA expression and in vitro leptin secretion are much greater in adipocytes isolated from subcutaneous adipose tissue than in those isolated from visceral adipose tissue (18, 19, 34); therefore, the contribution of visceral adipose tissue to whole body leptin metabolism is minimal compared with the contribution from subcutaneous fat tissue.

In summary, the normal decline in adipose tissue leptin production during early fasting is blunted in women with UBO and is responsible for their blunted decrease in plasma leptin concentrations. The altered decline in plasma leptin, in conjunction with an attenuated decline in plasma insulin (10), may be responsible for many of the alterations in the metabolic response to fasting associated with obesity.

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