Difference in leptin response to a high-fat meal between lean and obese men

P. IMBEAULT*, E. DOUCET*, P. MAURIÈGE†, S. ST-PIERRE*, C. COUILLARD†, N. ALMÉRAS‡, J.-P. DESPRÉS‡ and A. TREMBLAY*

*Division of Kinesiology, Department of Social and Preventive Medicine, Laval University, Ste-Foy, Québec, Canada G1K 7P4, †Lipid Research Center, Laval University Medical Research Center, Ste-Foy, Québec, Canada G1V 4G2, and ‡Québec Heart Institute, Laval Hospital Research Center, Ste-Foy, Québec, Canada, G1V 4G5

ABSTRACT

The aim of this study was to compare the leptin responses to a high-fat meal in lean and obese men, and to investigate whether the net leptin response (area under the incremental curve) after the meal was related to the thermic effect of food (TEF). Blood samples were collected after an overnight fast and every 2 h for 8 h after a high-fat breakfast (60 g of fat/m² body surface area) in 12 lean and 12 obese men for determination of glucose, insulin and leptin. The TEF was calculated as postprandial energy expenditure minus fasting energy expenditure, as measured by indirect calorimetry. Fasting plasma glucose levels were similar in lean and obese men, and increased in the same way after the meal. Fasting and postprandial plasma insulin concentrations were significantly greater in obese than in lean men (P < 0.01 and P < 0.05 respectively). Accordingly, obese men showed a significantly higher net insulin response than lean subjects (P < 0.001). Fasting plasma leptin levels were greater in obese than in lean men (P < 0.001). After the meal, plasma leptin increased significantly in lean men, whereas it decreased in obese men (group by time interaction, P < 0.01). The net response of leptin was greater in lean than in obese men, but this did not reach statistical significance (P = 0.07). Moreover, the TEF was similar in the two groups. No significant relationship was observed between either the net insulin response or the net leptin response after the high-fat meal and the TEF of lean subjects (−0.05 < r < 0.31). In obese men, the net response of insulin was correlated significantly with TEF (r = 0.70, P < 0.05), whereas the net response of leptin was not (r = −0.40). These results suggest that obesity is related to an impaired regulation of leptin by insulin, since leptin levels increased in lean men but decreased in obese men following a high-fat meal. Moreover, the fact that the postprandial leptin responses of both lean and obese men were not significantly related to the TEF suggests that the ob gene product is probably not acutely involved in the control of this energy expenditure component in humans.

INTRODUCTION

There is now convincing evidence that leptin acts as a signal reflecting acute variation in energy balance, rather than as an ‘adipostat’ [1]. Indeed, several studies have shown that plasma leptin levels decrease much more than would be expected on the basis of body fat loss [2,3]. The same phenomenon is observed with refeeding, as leptin increases in a disproportionate manner when compared with the increase in adipose tissue mass [4]. Taken

Key words: indirect calorimetry, leptin response, obesity, postprandial insulin.
Abbreviation: TEF, thermic effect of food.
Correspondence: Dr Angelo Tremblay (e-mail angelo.tremblay@kin.msp.ulaval.ca).
After voiding, the subjects rested comfortably in the supine position for 15 min, whereafter resting metabolic rate was measured for 15 min. Immediately after this, an intravenous catheter was inserted into a forearm vein and a blood sample was taken before the breakfast. The subjects were then offered a high-fat breakfast that was ingested within a maximum period of 30 min. After the meal, gas exchange was measured hourly (15 min measurement periods), and blood samples were taken every 2 h for an 8 h period. During the postprandial measurements, subjects were allowed to watch light entertainment movies. During the short breaks, the subjects were allowed to sit, walk quietly and go to the washroom.

### Total body fat and regional fat distribution

Body density was determined by the underwater weighing technique, and percentage body fat was derived from body density [18]. Pulmonary residual volume was measured using the helium dilution method [19]. Fat mass was calculated as total body weight minus fat-free mass. Computed tomography (CT) was performed with a Somatom DRH scanner (Siemens, Erlangen, Germany), according to the methodology described previously by Sjöström et al. [20]. Briefly, subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal level (between the L4 and L5 vertebrae), using an abdominal scout radiograph to establish the position of the scans to the nearest millimetre. Total adipose tissue areas were calculated by delineating the abdomen with a graph pen and then computing adipose tissue surfaces with an attenuation range of −190 to −30 Hounsfield units [21]. Abdominal visceral adipose tissue area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous adipose tissue area was calculated by subtracting the visceral adipose tissue area from the total abdominal adipose tissue area.

### Oral lipid tolerance test

All subjects were instructed to abstain from prolonged and vigorous physical activity for 2 days before the test meal. After a 12 h overnight fast, each participant was given a breakfast containing 60 g of fat/m² body surface area, as described previously [22]. This approach was chosen to represent the energy expenditure of each individual over the 8 h of the experimental session. The test meal had an energy content (mean ± S.D.) of 7464 ± 167 kJ (1784 ± 40 kcal) and 8485 ± 167 kJ (2028 ± 40 kcal) for the lean and obese men respectively, and consisted of 64% of energy as fat, 18% of energy as carbohydrate and 18% of energy as protein. The Canadian Nutrient File [23] was used in the calculation of the energy and nutrient composition of the breakfast. The meal was composed of eggs, cheese, toast, peanut butter, peaches, whipped cream and milk, and was well tolerated by all subjects.

**METHODS**

**Subjects**

Twelve healthy lean and twelve obese men were recruited through the media, and gave their written informed consent to participate in the study, which was approved by the Laval University Medical Ethics Committee. Men with a body fat percentage (estimated by hydrodensitometry) of < 25% were considered as lean, whereas a body fat percentage of > 30% defined the obese group. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus or endocrine disorders, or those on medication that could have influenced triacylglycerol metabolism (β-blockers, anti-hypertensive drugs, etc.), were excluded from the study. All participants were sedentary (no more than two continuous exercise sessions of 30 min per week), non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight-reducing program, and their body weight had been stable during the last 6 months prior to the study.

**Experimental protocol**

The subjects arrived at the laboratory at 06.45 hours. After voiding, the subjects rested comfortably in the
Postprandial leptin response and obesity

Subjects took approx. 25 min to consume the meal, beginning between 07.30 and 07.45 hours. After the meal, subjects were not allowed to eat for the next 8 h, but were given free access to water.

**Energy expenditure**

Resting energy expenditure was measured by indirect calorimetry after a 12 h overnight fast. After a 15 min resting period, expired gases were collected over 15 min through a mouthpiece while the nose was clipped. After the meal, respiratory gas exchanges were measured for 15 min periods every 1 h over the 8 h period. TEF was calculated as the the total increase in caloric expenditure above resting energy expenditure. Oxygen and carbon dioxide concentrations were determined by non-dispersive IR analysis (Uras 10 E; Hartmann & Braun), and pulmonary ventilation was assessed with an S-430A measurement system (Ventura) which has been validated previously by Linnarsson et al. [24]. The Weir formula [25] was used to determine the energy equivalent of oxygen volume.

**Concentrations of glucose, insulin, leptin and non-esterified fatty acids**

Plasma glucose levels were determined using the glucose oxidase assay [26] (Sigma, St. Louis, MO, U.S.A.). Plasma insulin concentrations were measured by a commercial double-antibody RIA (Linco Research, St. Louis, MO, U.S.A.) that shows little cross-reactivity (<0.02%) with pro-insulin [27]. Plasma leptin levels were determined with a highly sensitive commercial double-antibody RIA (Linco Research) that detects relatively low leptin levels (0.5 ng/ml) and does not cross-react with human insulin, pro-insulin, glucagon, pancreatic polypeptide or somatostatin. Plasma non-esterified (‘free’) fatty acid levels were measured by using a colorimetric method [28].

**Statistical analyses**

Student’s *t* test was used for comparisons between lean and obese groups. ANOVA for repeated measures was used to verify differences between groups (lean and obese) in plasma insulin, glucose and leptin concentrations, as well as energy expenditure. Identification of a significant group by time interaction led to further analysis of a simple main effect for group. The level of significance was set at *P* < 0.05. The areas under the incremental curve or the net responses for the 8 h measurement periods were calculated separately for each subject as the difference between the integrated area of the response curve and the rectangular area determined by the basal values. Negative areas were included. Associations between two variables were quantified by Pearson’s product–moment correlation coefficients. All analyses were performed using the Jump version 3.2.2 program (SAS Institute Inc., Cary, NC, U.S.A.) adapted for Macintosh computers.

**RESULTS**

Physical characteristics of subjects are presented in Table 1. Lean and obese men were of similar age. As expected, obese men were characterized by greater body weight, body mass index, fat mass, and subcutaneous and visceral abdominal adipose tissue areas than lean individuals (*P* < 0.001). No difference was found for fat-free mass between the groups.

Figure 1 shows plasma glucose, insulin and leptin concentrations before and after the high-fat meal in lean and obese subjects. Fasting plasma glucose concentrations were similar in the two groups (Figure 1A). A significant time effect for glucose was observed in both groups (*P* < 0.001). However, no difference in plasma glucose levels was found between groups during the meal. Also, the net response of glucose was similar in lean and obese men.

On the other hand, fasting plasma insulin levels were greater in obese than in lean subjects (*P* < 0.01) (Figure 1B). A significant group by time interaction (*P* < 0.05) was observed for plasma insulin levels, revealing that obese men had a significantly greater postprandial increase in insulin level than lean subjects. Accordingly, obese men showed a significantly higher net response than lean subjects for insulin (*P* < 0.001).

Fasting plasma leptin levels were significantly higher in obese than in lean men (*P* < 0.001) (Figure 1C). Moreover, the plasma leptin response pattern differed significantly between groups after the meal (group by time interaction, *P* < 0.01). Indeed, lean men showed a significant increase in plasma leptin levels after the meal, whereas leptin concentrations decreased significantly in obese subjects. The mean net response of leptin was positive in lean subjects and negative in obese men, but this difference fell short of statistical significance (*P* = 0.07). Postprandial levels of non-esterified fatty acids

### Table 1 Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean (n = 12)</th>
<th>Obese (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47 ± 10</td>
<td>46 ± 9</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>75 ± 8</td>
<td>100 ± 12*</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25 ± 2</td>
<td>33 ± 4*</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>22 ± 4</td>
<td>36 ± 3*</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>17 ± 4</td>
<td>37 ± 6*</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>59 ± 6</td>
<td>63 ± 8</td>
</tr>
<tr>
<td>Abdominal adipose tissue area (cm²)</td>
<td>183 ± 50</td>
<td>395 ± 105*</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral</td>
<td>112 ± 35</td>
<td>210 ± 85*</td>
</tr>
</tbody>
</table>
Figure 1  Plasma levels of glucose (A), insulin (B) and leptin (C) before and after a high-fat meal in 12 lean and 12 obese men
Data are means ± S.E.M. Bars represent the areas under incremental curves (net responses) of each group. (A) Significant main effect for time at $P < 0.001$. (B) Significant interaction between group and time at $P < 0.05$; significant difference between groups at $\&$, $P < 0.01$. (C) Significant interaction between group and time at $P < 0.01$; level of significance of difference between groups: $\&$, $P = 0.07$.

Figure 2  Energy expenditure before and after a high-fat meal in 12 lean and 12 obese men
Significant main effects for group ($P < 0.001$) and time ($P < 0.001$). Bars represent the areas under incremental curves (TEF) of each group. Data are means ± S.E.M.
Postprandial leptin response and obesity

Figure 3 Relationships between net insulin or net leptin response after a high-fat meal and the TEF in 12 lean and 12 obese men

Increased significantly in both groups (time effect, \( P < 0.001 \)), and were higher in the obese group (group effect, \( P < 0.05 \)) (results not shown).

As expected, basal resting metabolic rate was significantly higher in obese than in lean men \( (P < 0.001) \) (Figure 2). In both groups, a significant increase in energy expenditure occurred in response to the meal (time effect, \( P < 0.001 \)). Energy expenditure was higher in obese than in lean men over the meal (group effect, \( P < 0.001 \)), but the TEF (net response of energy expenditure) was similar in the two groups.

No significant relationship was observed between either the net insulin or the net leptin response to the high-fat meal and TEF in lean subjects \( (-0.05 < r < 0.31) \) (Figure 3). In obese men, the net response of insulin was correlated significantly with TEF \( (r = 0.70, P < 0.05) \), but the net response of leptin was not \( (r = -0.40) \).

DISCUSSION

The present study showed that the postprandial leptin response over an 8 h period following a high-fat meal in men differed significantly depending on the body composition. Indeed, lean subjects showed an increase in postprandial plasma leptin levels, whereas leptin concentrations decreased in obese individuals. Despite these divergent postprandial leptin responses, no difference was observed in the TEF between the two groups studied.

Previous studies have demonstrated that plasma leptin was increased by physiological insulin infusions in humans [13]. Our results in lean individuals are concordant with this observation, since their plasma insulin and leptin concentrations increased during the high-fat meal. Noteworthy is the fact that the increase in leptin in response to the test meal in lean men was delayed compared with that of insulin. These results confirm those of Dallongeville et al. [5], who showed that the serum leptin response of healthy men to a fatty meal only began to reach statistical significance 6 h after the meal. To some extent, these results support the notion that the postprandial leptin response could be a consequence of the effect of insulin on adipocytes, as suggested previously [29]. Our study also revealed significantly reduced leptin production in obese men, despite the fact that these subjects displayed significantly increased insulin secretion after the high-fat meal. This is concordant with the findings of Pratley et al. [6], who showed that...
plasma leptin concentrations in obese subjects fell by 8% below baseline 2–4 h after a mixed meal. A physiological explanation for this observation is a proposed continuation in the decline of leptin from the previous night’s acrophase [12]. Whether or not a longer post-prandial period would have been required to observe a rise in serum leptin levels in obese men is not known.

Although leptin has been found to increase energy expenditure in ob/ob mice [15–17] and in non-obese mice [15], such observations remain to be confirmed in humans. In the present study, no significant relationship was observed between the TEF and the net response of leptin in either group of men. This observation thus suggests that leptin does not modulate this component of energy expenditure in humans. According to the current literature, the contribution of the ob gene product to human energy expenditure appears to be involved in the fall in resting energy expenditure accompanying weight loss. Indeed, Wadden et al. [30] observed that the decrease in resting energy expenditure was correlated significantly with changes in plasma leptin after 40 weeks of energy restriction in obese women. We have also recently reported that changes in resting energy expenditure of obese men in response to weight loss were best predicted by changes in plasma leptin, even after adjusting for changes in body composition [31].

As expected, the obese group showed a greater insulin response after the meal than lean subjects. Based on previous studies showing that men with low insulin sensitivity displayed an impaired TEF [32], it would have been expected that obese men showed a lower TEF in response to the high-fat meal. However, one should keep in mind that hyperinsulinaemia with euglycaemia has been shown previously to increase muscle sympathetic nervous activity [33], a well-known predictor of energy expenditure [34]. In this regard, the significant positive relationship observed between the net insulin response and diet-induced thermogenesis in obese men suggests that insulin is an important factor involved in the control of TEF. To some extent, these results re-emphasize the notion that insulin exerts a regulatory role, promoting the restoration of energy balance when one gains weight [35].

In addition to the fact that leptin administration increases energy expenditure in mice, there is also evidence that the administration of the ob gene product has an inhibitory effect on food intake [15–17]. In this regard, it would have been interesting to verify whether the different pattern of leptin responses observed in lean and obese men after the high-fat meal influenced their satiety and hunger ratings differently. One could suggest that obese men might have felt more hungry or less full than their lean counterparts, since they had reduced leptin secretion following the meal. However, the association between leptin levels and hunger and satiety seems to occur more on a long-term than on a short-term basis in humans. Indeed, some studies reported that leptin is a physiological regulator of hunger during energy deficit, since the greatest increase in hunger rating coincided with the largest percentage fall in circulating leptin levels in response to a moderate energy deficit in women [36,37]. Moreover, postprandial hunger and satiety ratings do not seem to be related to plasma leptin levels following a meal, at least in lean men [7,8]. Further studies should nevertheless be performed to examine whether the different patterns of leptin response between lean and obese individuals following a high-fat meal could have a different impact on their appetite control.

In summary, lean and obese men presented different patterns of leptin response over an 8 h period after the consumption of a high-fat meal: lean men showed increased leptin levels, whereas obese men displayed decreased postprandial lepinenaemia. Moreover, the absence of a significant relationship between the post-prandial leptin response and TEF in both lean and obese men suggests that this hormone is not acutely involved in the regulation of this component of energy expenditure in humans.

ACKNOWLEDGMENTS

We express our gratitude to Dr Gilles Lortie for medical supervision of the subjects. Linda Drolet and Alfred Breton-Paré are also gratefully acknowledged for their technical assistance. P. I. is the recipient of a Natural Sciences and Engineering Research Council of Canada fellowship. This research was supported by the Medical Research Council of Canada and the Fonds FCAR–Québec.

REFERENCES