Leptin response to carbohydrate or fat meal and association with subsequent satiety and energy intake

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IT IS NOW WELL ESTABLISHED that high-fat diets are associated with less efficient energy compensation than high-carbohydrate diets (26). The physiological determinants of this association are not well-known, and it is not clear whether leptin has a role in this process. In rodents, food ingestion increases leptin (ob) gene expression and/or plasma leptin levels, suggesting a link between nutrient balance and the leptin regulatory pathways (29). In humans, early studies failed to detect any acute effect of food intake on postprandial leptin response (7, 32). Later, Kolaczynski et al. (18) reported that overfeeding resulted in an increase in serum leptin, which peaked between 5 and 10 h after the overfeeding program was started. Consistent with this observation, Schoeller et al. (30) found that the diurnal rhythm of plasma leptin in young men is regulated by meal timing. Finally, Dallongeville et al. (9) found an increase in leptin levels starting 4 h after a normocaloric mixed meal, indicating that food intake stimulates leptin secretion in humans. The mechanism of this effect is not known. Insulin, which stimulates leptin secretion in cachexia interventions, may play a physiological role in this process (3). In that case, carbohydrate intake could be a determinant of leptin response. Consistent with this hypothesis, Jenkins et al. (13) have observed that during caloric restriction changes in leptin levels are correlated with changes in carbohydrate intake, but they did not study hunger and satiety.

Previous experiments have shown that administration of leptin causes an acute decrease in food intake in rodents (2, 6), indicating that leptin has an inhibitory effect on food intake. In humans, few studies have examined the effect of leptin on satiety. The results of these studies have not confirmed this hypothesis (14, 15, 33). Recently, circulating leptin was independently related to hunger during long-term energy deficit (16).

The aim of the present study was to test whether the macronutrient content of the meal could influence postprandial leptin response. We compared the leptin response to two isoenergetic meals with different amounts of carbohydrate and fat but similar amounts of protein. We also examined if, in these conditions, leptin levels were associated with postprandial satiety and hunger and subsequent food intake.

METHODS

Subjects. Eleven men and eleven women were recruited through posters in the Université de Lille II community. They completed a questionnaire about health status, eating, drinking, and exercise habits, weight history and weight concern. The subjects included in the study were healthy nonsmokers; their weight was stable during the 6 mo preceding the study, without food restriction or dieting. Subjects with a body mass index above 27 kg/m² and/or antecedents of obesity were excluded from the study. The protocol was approved by the Hospital Ethics Committee (Centre Hospitalier Regional Universitaire de Lille), according to current French regulations.

Food and macronutrients. Test meals: the two test meals were composed of either cream or fat-free cottage cheese, vanilla or coffee flavored, according to the subject’s taste (Table 1). The energetic value was identical for men and women (3.4 MJ). Subsequent meal: the subsequent meal, a self-selection buffet, allowed ad libitum consumption of a variety of foods. All foods were weighed once before being served and again after the subject had finished eating to obtain the amount consumed of each food.

Ratings. Subjects rated their hunger and satiety on a 100-mm visual analog scale (VAS) on which the most positive and the most negative ratings were indicated at each end. Subjects completed baseline ratings at the beginning of each session and after the test meal. Ratings were then completed each hour for 9 h and before and after the second test meal.
Table 1. Ingredients, macronutrients, and energy content of test meals

<table>
<thead>
<tr>
<th>Ingredients, g</th>
<th>Carbohydrate Meal</th>
<th>Fat Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-free cottage cheese</td>
<td>500</td>
<td>75</td>
</tr>
<tr>
<td>Protifar Plus*</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Aspartame/cream mix</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Strawberry jam</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Maltose solution</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Total energy, kJ</td>
<td>3,448</td>
<td>3,373</td>
</tr>
<tr>
<td>Energy from fat, % energy</td>
<td>38</td>
<td>2,671</td>
</tr>
<tr>
<td>Saturated fatty acid (%)</td>
<td>94.5</td>
<td>94.5</td>
</tr>
<tr>
<td>Monounsaturated fatty acid (%)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Polyunsaturated fatty acid (%)</td>
<td>5.5</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Biochemical and clinical measurements. Blood was collected in dry tubes and allowed to clot for 1 h. Serum was separated by centrifugation (2,500 rpm) for 20 min at 4°C. Glucose (glucose hexokinase method, Randox Laboratories, Antrim, UK) and triglycerides (triglycerides GPO-PAP, Boehringer Mannheim, Mannheim, Germany) were determined enzymatically. Hormone levels were determined with commercially available immunological assays. Insulin was measured by RIA (Bi-l-Insulin RIA, ERIA Diagnostics, Pasteur, France). C peptide levels were determined by RIA (RIA-coat C-Peptid, ByK-Sangteg Diagnostica, Dietzenbach, Germany). Leptin levels were measured in duplicate by RIA (human leptin RIA kit, Wako-Chemie Medical, Bad-Homburg, Germany). The lower limit of sensitivity of the assay is 0.5 ng/ml. For values between 1.3 and 1.9 ng/ml the intra- and interassay variability was 3.7 and 12.6%, respectively. Very low-density lipoproteins (VLDL) were separated by ultracentrifugation, with a Beckman TL100, from 0.5 ml of serum by a single spin at a density of 1,006 g/ml with modifications; 0.5 ml of 0.9% NaCl was added to 0.5 ml of serum and spun in a polycarbonate tube (400,000 g, 20°C) in a Beckman 100.2 Ti rotor for 3 h. The tube was cut in two parts, and the remaining 0.5-ml infranatant was analyzed for lipids. Body fat content was assessed by two-frequency (5 and 100 kHz) bioimpedance analysis (Analyzer 2, Spengler, France).

Experimental protocol. The experiment used a within-subject repeated-measure design, in which each subject served as his/her own control. There were three subsets of experiments: carbohydrate meal, fat meal, and no meal. The order of presentation was balanced across subjects. There was no evidence of a statistically significant effect of order of the experiments on biological responses to the meal. The level of statistical significance was set at $P < 0.05$. Responses to test meals were estimated as the postprandial area under the curve (AUC) and calculated as follows: $AUC = \frac{1}{2} (T_9 - T_0)$, in which $T_0$ varies from T0 to T9. There was no evidence of a statistically significant effect of order of the experiments on biological variables.

RESULTS

The anthropometric characteristics of the subjects are presented in Table 2. Mean weight ($P = 0.003$), height ($P = 0.005$), and fat-free mass ($P < 0.0001$) were higher in men than in women.

Baseline biological values. For baseline assessment of biological variables, blood samples were drawn three times at an average interval of 1 wk (before each test meal session). Because mean serum insulin and leptin concentrations were higher in females than males, the statistical analyses were performed separately for each gender. Baseline serum glucose, C peptide, insulin, and leptin concentrations were not significantly different before the various test meals in men and women (Table 3).

Test meals. To test whether the macronutrient composition of a meal influences the postprandial leptin response, each participant consumed, in a random order, an isocaloric test meal (high carbohydrate or high fat) or remained fasting. A two-way ANOVA with repeated measures showed an interaction between the tests (carbohydrate meal/fat meal/fast) and the postprandial interval (T0-T9 h) for serum glucose, insulin, C peptide, and leptin in both male and female groups (Fig. 1, data for glucose and C peptide are not shown).

Table 2. Characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.9 ± 3.2</td>
<td>21.5 ± 1.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.5 ± 8.5</td>
<td>58.4 ± 6.0</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.77 ± 0.1</td>
<td>1.64 ± 0.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.3 ± 1.8</td>
<td>21.6 ± 1.8</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>60.2 ± 6.0</td>
<td>44.2 ± 4.2</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>9.3 ± 5.7</td>
<td>14.2 ± 5.3</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n = 11$ in men and 11 in women. BMI, body mass index.
Post hoc analysis indicated that postprandial serum glucose, insulin, and C peptide levels were significantly higher, usually between 1 and 4 h, after the high-carbohydrate meal than during the fasting experiment. In men, postprandial serum insulin and C peptide levels were only marginally significantly higher after the high-fat meal than during the fasting experiment. The pattern of postprandial serum leptin levels was different from that of serum glucose, insulin, and C peptide levels. Early (1–3 h) after food consumption, no statistically significant difference was observed in leptin levels in the test meal group. Leptin levels started to diverge 4 h postprandially. In both genders, leptin levels were higher between 4 and 9 h after the carbohydrate meal than after the fat meal and during fasting. In women, postprandial leptin concentrations were also higher between 5 and 9 h after the fat meal than during the fasting experiment, whereas in men no difference in postprandial leptin levels after the high-fat meal and during the fasting experiment could be demonstrated.

Correlation analysis. To evaluate the possible association between leptin response and other biological variables, an analysis was carried out on a possible correlation between leptin response and glucose, VLDL-triglyceride, insulin (Fig. 2), and C peptide responses. Leptin response was significantly correlated to C peptide ($r = 0.55; P < 0.0001$) and insulin ($r = 0.51; P < 0.0001$) responses but not to glucose and VLDL-triglyceride responses.

Hunger and satiety. To assess whether hunger or satiety was related to leptin response, both were measured every hour after food intake, with a visual analogical scale. This method yields data with important interindividual variability that could reduce the statistical power to uncover subtle differences among meals. Despite this variability, two-way ANOVA showed a statistically significant interaction between test (carbohydrate meal/fat meal/fast) and postprandial interval (T0-T9 h) for hunger and satiety ratings. In women, between 1 and 9 h postprandially, both high-fat and

### Table 3. Baseline Values

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemia, g/l</td>
<td>Fast</td>
<td>Fat meal</td>
</tr>
<tr>
<td>C peptide, ng/ml</td>
<td>1.47 ± 0.25</td>
<td>1.33 ± 0.40</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>4.34 ± 2.53</td>
<td>4.67 ± 2.45</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>3.7 ± 2.6</td>
<td>3.3 ± 2.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. CHO, carbohydrate meal; NS, not significant. P values of one-way ANOVA with repeated measures.

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**Fig. 1.** Mean concentrations of insulin (A and C) and leptin (B and D) according to length of fasting experiment and after food intake in women (A and B) and men (C and D). Carbohydrate (CHO), fat, and fasting experiments are represented by circles, squares, and triangles, respectively. ANOVA with repeated measures indicates a statistically significant interaction between test (carbohydrate meal/fat meal/fast) and postprandial intervals (T0-T9) for insulin and leptin in both men and women. Post hoc analysis: different from corresponding point in time during fast $P < 0.01$ (a) and $P < 0.001$ (b); different from corresponding point in time after fat meal $P < 0.01$ (c) and $P < 0.001$ (d).
high-carbohydrate meals were associated with significantly greater ratings of satiety and lower ratings of hunger than during the fasting experiment. There was no evidence of a statistically significant difference in hunger and satiety between carbohydrate and fat meals in women (Fig. 3). In men, both carbohydrate and fat meals were associated immediately after food intake with higher and lower ratings of satiety and hunger, respectively, than during the fasting experiment. However, from the third and fourth hours to the end, hunger and satiety scores were similar in the carbohydrate meal and fasting experiment. Whereas satiety ratings were higher 3 and 4 h after the fat meal than after the carbohydrate meal, hunger ratings were lower 6 and 7 h after the high-fat meal than after the carbohydrate meal (Fig. 3).

Subsequent food intake. Although there was a tendency to lower intakes of energy after the carbohydrate meal, this difference did not reach the level of statistical significance. This decreased energy intake was related to a reduction in carbohydrate intake (Fig. 4).

**DISCUSSION**

This study was designed to test whether the macronutrient content of the diet affects postprandial leptin...
secretion and whether the leptin response has an impact on hunger, satiety, and spontaneous food intake. Its major findings are 1) that a carbohydrate meal induces a greater postprandial leptin response than an isonenergetic fat meal, 2) that this response is correlated to the physiological postprandial insulin response, and 3) there is no evidence of an association among postprandial leptin response, postprandial satiety or hunger, and subsequent food intake. These findings suggest that at a steady energetic state, postprandial leptin secretion is mainly modulated by the carbohydrate-insulin pathway and that leptin levels do not explain short-term food intake regulation.

The carbohydrate meal stimulated the postprandial leptin response more than the fat meal. This response was progressive and started to diverge from that of fat meal and of fast approximately 4–5 h after food ingestion. These results are in agreement with our previous study showing that the postprandial leptin response to a meal is delayed to 4 h after the meal (9). Other investigations support the role of carbohydrates in the regulation of serum leptin levels. First, glucose infusion prevents the serum leptin decrease associated with prolonged fast (4). Second, serum leptin and insulin levels are correlated to changes in the dietary carbohydrate intake during caloric restriction (13). Finally, the mean daily levels, as well as the nocturnal rise, of leptin secretion are higher after high-carbohydrate/low-fat meals than after low-carbohydrate/high-fat meals (11). Altogether, these findings suggest that carbohydrate feeding has an important role in the regulation of leptin levels.

In this study, a correlation was found between insulin and leptin postprandial responses to food intake. This suggests that the postprandial effect of carbohydrate intake on leptin levels is, at least partially, mediated by insulin. Several cross-sectional studies have shown an association between fasting leptin and insulin levels (24, 27). In contrast, conflicting results have been published concerning the effects of insulin infusion on leptin secretion. Earlier investigations with short-term insulin infusions failed to detect any effect of insulin (8). In contrast, 24- to 72-h insulin infusions (3, 17) or short-term high-concentration insulin infusions cause a significant increase in leptin (23, 35). Saad et al. (28) have recently demonstrated that physiological insulinemia acutely increases leptin levels, in comparison with saline infusion during which leptin levels decrease progressively. These authors underlined the fact that earlier studies could not detect an acute effect of physiological insulin levels on leptin because they had overlooked the decline of leptin during fasting. The present study demonstrates that the leptin response to a meal is related to the physiological insulin response. The delay between leptin and insulin response is in agreement with in vitro experiments that provided evidence that leptin secretion is directly proportional to glucose uptake and metabolism in adipocytes (25).

In women, postprandial leptin levels were higher after the fat meal than during the fasting experiment. This effect reached statistical significance in women but not in men. This gender difference may be the consequence of the relatively higher energy content of a meal in women (38% of theoretical energy needs) compared with men (30%). On the other hand, the higher levels of circulating leptin in females may facilitate the discrimination between fast and a fat meal. Because fat ingestion had no significant impact on insulin secretion, these results suggest that factors other than insulin explain the difference in postprandial leptin variation between fat meal and fasting. In this respect, the energy load of the fat meal could play...
an independent role in the regulation of leptin secretion. It has been suggested that leptin acts as a sensor of energy balance (20). An energy deficit induced either by fasting (37) or an increased energy expenditure, such as a marathon run (19), acutely contributes to decreased leptin levels, independently of changes in the fat mass. Finally, Wang et al. (36) demonstrated that leptin concentration is influenced by a nutrient-sensing mechanism; they found in vitro that the leptin gene expression was related to the activity of the hexosamine biosynthetic pathway, a cellular sensor of energy availability. Our results support the concept that the energetic content of a meal regulates the short-term secretion and levels of leptin independently of insulin.

We also aimed at investigating the possible role of leptin in the regulation of satiety and hunger after food intake. We could not demonstrate such a role immediately after food intake. This lack of association is probably due to the fact that satiety, hunger, and food intake are determined by several mechanisms active at different times. Immediately after a meal (1–4 h), gastrointestinal responses overcome other regulatory factors. At this stage, leptin levels did not differ in the various test meal conditions, whereas satiety and hunger ratings were higher and lower, respectively, in both meal conditions compared with fast. After the fourth hour, postprandial leptin levels started to diverge from those of fast; however, in women, hunger and satiety ratings converged among test meals. These findings are in agreement with short-term studies in humans where no relationship between leptin levels and hunger was found (14, 15, 33). In contrast, long-term studies have shown that fasting leptin levels are associated with decreased hunger (12) and increased satiety (16) during energy restriction. Therefore, the association between leptin levels and hunger and satiety seems to be delayed.

The impact of leptin levels on spontaneous food intake has not been explored in humans. Nine hours after the meal, food intake was not significantly different after the carbohydrate or fat meal nor was leptin concentration inversely correlated to food intake, suggesting that in humans leptin has no impact on the short-term regulation of food intake (next meal). These results are consistent with the effects of leptin on food intake in animals. In rodents, food intake was reduced after 4 h of an intraperitoneal infusion of leptin (1), and in monkeys the central administration of recombinant leptin had no acute effect but reduced food intake the following day (34). These studies suggest that enhanced leptin secretion is more closely related to long-term (days) than to short-term (h) food consumption.

In conclusion, the results of the present study demonstrated that a carbohydrate meal induces a greater postprandial leptin response than an isonenergetic fat meal and that this response is correlated to the physiological postprandial insulin response. It has recently been demonstrated that weight loss was more strongly associated with change in percent energy from fat than with change in total energy intake (31) while the fat-free mass was maintained (22). The relationship between leptin levels and carbohydrate intake suggests that leptin could contribute to the beneficial impact of carbohydrate-rich diets. Long-term studies are necessary to confirm this hypothesis.

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