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Serum leptin is associated with the perception of palatability during a standardized high-carbohydrate breakfast test

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Key words: breakfast, eating disorders, insulin, leptin, palatability.

Abbreviations: BMI, body mass index; CV, coefficients of variation; IRI, insulin resistance index.

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Leptin is an adipocyte-derived signalling molecule which plays a key role in the regulation of body weight and energy expenditure. Since its involvement in human eating behaviour is still poorly understood, we investigated whether the perception of palatability of food was related to fasting serum leptin levels. Twenty-six non-diabetic subjects, six men and twenty women of widely ranging age and body mass index, performed a standardized high-carbohydrate breakfast test. Palatability was evaluated with a visual analogue scale, body composition by bioelectrical impedance, serum leptin and plasma insulin by radioimmunoassay. Palatability was correlated to fasting serum leptin levels independently of body mass index, body fat mass and percentage of body fat ($P < 0.01$). No significant relation was observed with peaks of insulinaemia, integrated concentrations of insulin or insulin resistance indices. A stepwise regression analysis indicated that serum leptin gave the strongest predictive association with palatability. These results suggest that the leptin system may be involved in the regulation of human eating behaviour in relation to the perception of palatability of food.

INTRODUCTION

Leptin, the adipocyte-derived product of the obese (*ob*) gene, is currently believed to play a key role in the regulation of body weight, food intake and energy expenditure. In *ob*/*ob* mice, mutations in the *ob* gene prevent normal leptin production and cause overeating and obesity. Treatment of these animals with recombinant leptin decreases food intake and body weight, and increases thermogenesis [1]. Therefore, leptin appears to act as an afferent satiety signal in a feedback loop affecting satiety centres of the brain, probably through a number of hypothalamic mediators, including neuropeptide Y and corticotrophin-releasing factor [2]. In humans, complete leptin deficiency, i.e. the counterpart of *ob*/*ob* mice, has not yet been described. Only a few obese patients have been reported to have extremely low circulating concentrations of leptin: since no evidence of down-regulating factors was found, it was concluded that the production *per se* was disturbed [3]. In most obese subjects, leptin levels are high and correlate with the body mass index (BMI), the percentage of body fat [4,5] and the visceral or subcutaneous fat area [6]. This increase in serum leptin is thought to reflect a state of leptin resistance and result potentially from defective receptor or post-receptor transducing mechanisms, or reduced transport into the cerebrospinal fluid [7,8].

The role of leptin that has attracted more attention is the regulation of energy balance. In contrast, its involvement in human eating behaviour is still poorly understood. The highly complex system that regulates feeding integrates physiological stimuli, environmental information such as sight or smell of food, and conditioned behaviour. These various factors stimulate autonomic responses called cephalic-phase responses, e.g. insulin or gastric acid secretion [9]. The importance of human eating behaviour could be assessed in relation to the adjustments in energy expenditure. In a recent paper, it was concluded that serum leptin had no significant part in the short-term regulation of eating in obese women, since it was associated with neither the feeling of hunger nor with the will of eating [10]. We are not aware of any study on the relationships between leptin and palatability. Palatability, or taste pleasure, is supposed to be an important factor that regulates food intake. Although numerous studies have demonstrated that various substances, including neurotransmitters and neuromodulators, affect feeding behaviour, our knowledge about mechanisms underlying palatability and its involvement in energy balance is quite limited. Previous experiments in humans [11] or animals [12,13] have reported that a large part of postprandial thermogenesis was due to sensory stimuli induced by palatable food, accompanied by a marked activation of the sympathetic nervous system [14]. It was even proposed that the palatability of food was responsible for the diet-induced thermogenesis rather than the composition of the diet or the amount of the calories consumed [15]. Moreover, palatability was supposed to improve early insulin release after a meal in normal weight subjects [16]. Insulin could regulate the secretion of leptin, even if the reports to date have been contradictory [6]. Therefore, this study was undertaken in order to investigate the relationships between leptin, insulin and palatability during a standardized breakfast tolerance test, in a random sample of patients with a wide BMI range.

SUBJECTS AND METHODS

Subjects

Twenty-six patients (six male, twenty female), who came to our unit for a nutritional check-up, in which the breakfast test was used to assess glycoregulation, were recruited at random for the study. The subjects were non-diabetic and had no family history of diabetes mellitus.

Mean age was 39.4±2.1 (18–61) years [mean±S.E.M. (range)], weight 83.1±4 (53.8–119) kg, height 1.64±0.13 (1.51–1.85) m, waist-to-hip ratio 0.84±0.02 (0.60–1.10), BMI 31.2±1.5 (22.6–47) kg/m^2, body fat mass 33.5±3.4 (10–65.6) kg and percentage of body fat 38.1±2.2 (13.7–55.2)%. Thus, this study included a large number of patients who were clinically obese (16 subjects with BMI > 30 versus 2 subjects with BMI between 25 and 29.9 and 8 subjects with BMI < 24.9). Systolic blood pressure was 121.1±2.1 (100–140) mmHg and diastolic blood pressure 70.6±1.8 (60–90) mmHg.

No medication was taken on a regular basis. The subjects filled in a dietary questionnaire so that energy deficits could be excluded. The study was conducted in accordance with the Declaration of Helsinki (1989). Written informed consent was obtained from each patient after the protocol had been approved by the local Ethics Committee.

Anthropometry

Weight and height measurements were performed and BMI was calculated as weight in kilograms divided by height in metres squared (kg/m^2). Waist and hip circumference measurements were taken using a non-extensive flexible tape at the narrowest part of the torso and at the point of maximum extension of the buttocks respectively. The waist-to-hip ratio was then calculated.

Impedance in body tissues to the flow of an applied alternative current was measured by bioelectrical impedance analysis and the values obtained were used to estimate body composition (body fat mass, percentage of body fat). All bioelectrical impedance measurements were performed by a multi-frequency (1, 5, 10, 50, 100 kHz) device (Human IM-Scan from Dietosystem, Milan, Italy). We have previously evaluated the mean repeatability of this technique. Mean coefficients of variation (CV) for electrical values ranged between 0.8 and 4.2% whereas they were from 0.2 to 0.9% for the derived parameters of body composition. These data suggest that the reliability of the measurements is satisfactory [17].

High-carbohydrate breakfast tolerance test [18] and assessment of palatability [19,20]

No dietary restriction was imposed. However, patients were asked to fast for 12 h before starting the test at 08.30 hours. A cannula for blood sampling was set in the cephalic vein at the level of the cubital fossa. The subjects ate a standardized breakfast which was composed of bread (80 g), butter (10 g), jam (20 g), skimmed concentrated milk (80 ml) (Gloria SA, Paris, France), sugar (10 g) and powdered coffee (2.5 g). This breakfast thus comprised 2070 kJ with 9.1% protein, 27.5% lipid and 63.4% carbohydrate. The average time taken to consume the meal was 6 min.

The palatability was assessed with a 14-cm visual analogue scale, ranging from 'extremely unpleasant' to 'extremely pleasant'. Just after eating, subjects were asked to mark a vertical line on the scale. For data analysis, the centre of the scale was considered as the zero value (neutral) and scores of palatability were measured as deviations in centimetres from the zero point. Thus the scores ranged from -7 (distaste) to +7 (extreme pleasure). In a previous study concerning the satiating power of sweet caloric or non-caloric solutions, Rogers et al. [20] provided validation of visual analogue scales under control conditions. Additionally, we studied the reproducibility of our method in 10 healthy, normal weight, young men who tested

the breakfast twice with a 1-week interval. Mean CV for palatability scores ranged between 0.36 and 1.1%.

Blood samples were taken twice before the meal and at 15, 30, 60, 90, 120, 150, 180 and 210 min after the start of the meal. The volume of blood taken at each time did not exceed 10 ml. A preliminary blood glucose evaluation at each time was made with a glucose analyser (One Touch Profile from Lifescan, Issy-les-Moulineaux, France). An investigator remained with the patients during the test.

Biochemical analyses

Blood was kept on ice until centrifugation at 4 °C and the plasma or serum samples were stored at -80 °C until analysis. All samples were analysed for plasma insulin by radioimmunoassay (kit Insik-5 from Sorin Biomedica France, Anthony, France) and for plasma glucose with a Vitros Product Chemistry analyser (Johnson & Johnson Clinical Diagnostics, Rochester, NY, U.S.A.). The within-assay CV for insulin ranged from 6.2% (low values) to 10.6% (high values) and the between-assay CV from 6.6% (low values) to 10.8% (high values). The sensitivity (lowest detectable value) was 2 μ-units/ml.

Basal serum leptin concentrations (at time 0, just before the meal) were determined by a radioimmunoassay using a polyclonal antibody raised in rabbits against highly purified recombinant human leptin (Linco Research Inc., St. Louis, MO, U.S.A.) [21]. The within-assay CV ranged from 3.4% to 8.3%, and the between-assay CV from 3.6% to 6.2%; the sensitivity was 0.5 ng/ml.

Homoeostasis model assessment

The homoeostasis model assessment was used to evaluate insulin sensitivity. The corresponding insulin resistance index (IRI) is defined from baseline insulin and glucose values as insulin/(22.5$e^{-\ln \text{glucose}}$) [22], which rearranges into (insulin×glucose)/22.5.

Statistical analyses

The results are given as means±S.E.M., followed by range in parentheses. Statistical significance was set at $P < 0.05$. The normal distribution of the variables was checked with the Kolmogorov–Smirnov test. If the variables were not normally distributed, they were ln-transformed before analysis. Relationships between leptin, palatability and the other measurements were analysed using Pearson and partial correlation coefficients. Stepwise regression analysis was applied to select determinants of palatability. All calculations were performed with the SigmaStat package (Jandel Scientific, Erkrath, Germany).

RESULTS

Response to the breakfast tolerance test

Figure 1 shows the glycaemic and insulinaemic responses to the breakfast tolerance test, compared with the corresponding normal control ranges established in our unit [18]. The mean peak of glycaemia was 7.2±0.2 (4.6–9.8) mmol/l, integrated concentration of glucose 5.7±0.3 (3.9–9.4) mmol/l, peak of insulinaemia 77.7±10.8 (20–152) μ-units/ml and integrated concentration of insulin 53.2±6.8 (20.5–128.8) μ-units/ml.

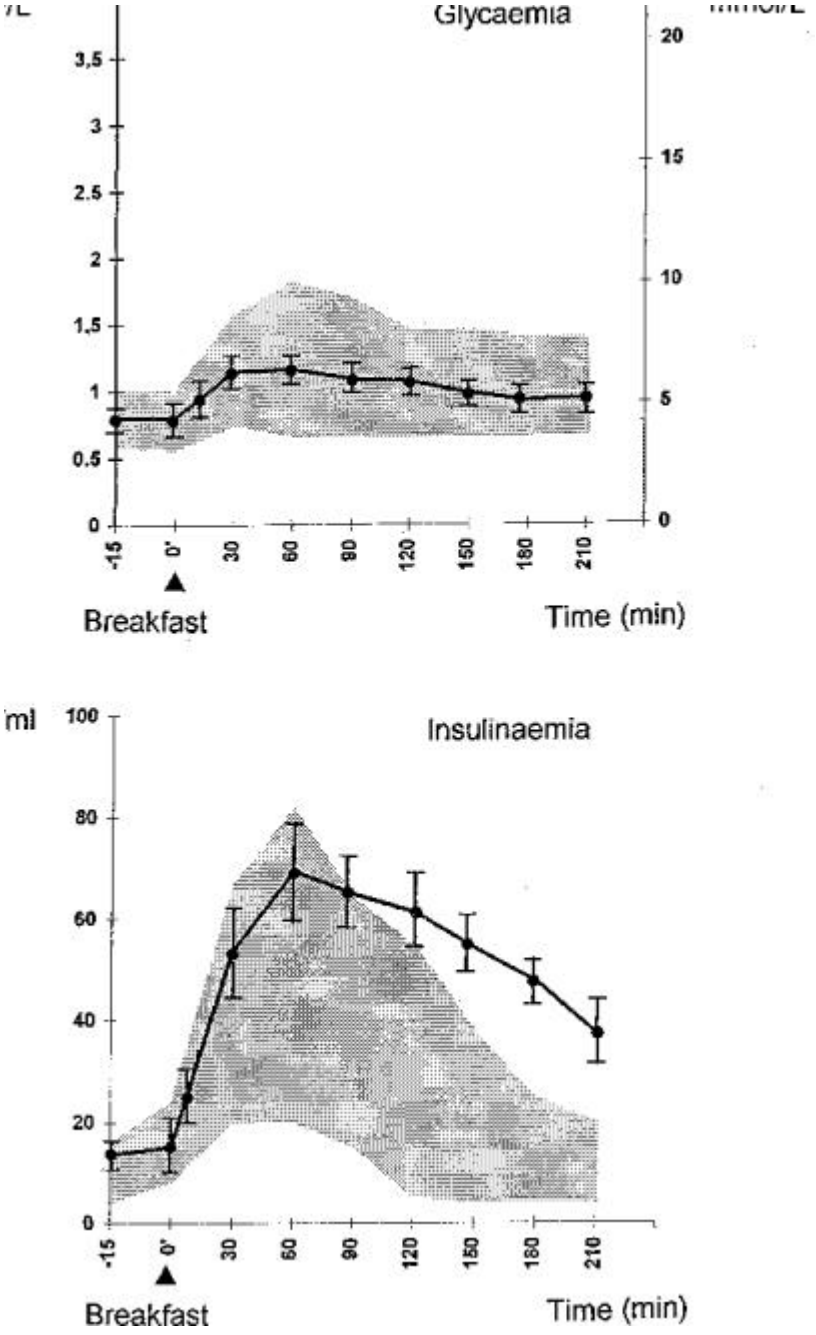

Figure 1 Glycaemic and insulinaemic responses to the breakfast tolerance test. Values are means±S.E.M. Corresponding reference ranges are given in grey.

Homoeostasis model assessment

The mean IRI was 2.50±0.34 (0.9–7.7).

Basal serum leptin levels and perception of palatability

The mean basal serum leptin concentration was 27.1±3.9 (2–74.7) ng/ml. As expected, fasting serum leptin was correlated with BMI, body fat mass and percentage of body fat (Table 1). The mean score of palatability was found at 3.5±0.5 and ranged from -2.6 to 7. Palatability

was correlated with fasting serum leptin ($r = 0.73$, $P < 0.0001$) (Figure 2), BMI ($r = 0.57$, $P = 0.003$), body fat mass ($r = 0.57$, $P = 0.003$) and percentage of body fat ($r = 0.56$, $P = 0.004$). No significant correlation was found between palatability and integrated concentration of glucose ($r = 0.05$, $P = 0.81$), peak of insulinaemia ($r = 0.33$, $P = 0.1$), integrated concentration of insulin ($r = 0.28$, $P = 0.16$) and IRI ($r = 0.23$, $P = 0.26$). A stepwise regression analysis showed that the prime determinant of palatability was basal serum leptin level ($P < 0.0001$): the above-mentioned factors did not significantly add to the ability to predict palatability.

Table 1 **Correlation coefficients for the association of the different parameters with fasting serum leptin**

HOMA, homoeostasis model assessment. Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.0001.

Parameters	Correlation coefficients	Partial correlation corrected for BMI	Partial correlation corrected for body fat mass	Partial correlation corrected for % body fat
Palatability	0.73***	0.55**	0.55**	0.57**
Peak of insulinaemia, ln transformed (μ-units/ml)	0.47*	0.31	0.26	0.19
Integrated concn. of insulin, ln transformed (μ-units/ml)	0.45*	0.21	0.21	0.19
IRI (HOMA, ln transformed)	0.44*	0.08	0.07	0.12
BMI (kg/m^2)	0.82***	–	–	–
Body fat mass (kg)	0.84***	–	–	–
Percentage of body fat	0.84***	–	–	–

Partial correlation analysis between serum leptin and the other parameters

Coefficients of correlation for the association of leptin with palatability, peak of insulinaemia, integrated concentration of insulin, IRI, BMI, body fat mass and percentage of body fat are given in Table 1. Since they were not normally distributed, values for peak of insulinaemia, integrated concentration of insulin and IRI were ln-transformed before analysis. When corrected for the influence of BMI, body fat mass or percentage of body fat, the partial

correlation coefficients became non-significant, except for the association between leptin and palatability.

DISCUSSION

In this study, we have described a significant relationship between fasting serum leptin levels and the perception of palatability during a breakfast tolerance test. The standardized meal was derived from that developed by Lefèbvre and Luyckx [23]. We introduced some modifications in order to mimic French nutritional habits: in particular, the usual French breakfast is mostly composed of carbohydrates. The quantity of carbohydrate (76 g) was chosen in order to obtain a similar increase in glycaemia as during a standard 75-g oral glucose tolerance test. We previously reported that this breakfast tolerance test, in obese subjects, gave similar information to the oral glucose tolerance test, but it is a more physiological procedure, which does not suppress the psychological and sensorial background of normal meals [24]. Thus we postulate that this test is appropriate for studying the relationships between palatability and feeding-induced metabolic responses in humans. The question of the validity of measurements obtained with rating scales must be discussed. These methods appear to have satisfactory validity, as reported by Rogers et al. [20]. We observed a good reproducibility of palatability scores despite a potential variability in ratings due to either methodological or biological day-to-day variations in subjective feeding sensations. This could be explained at least in part by the subjects' prior experience of the breakfast.

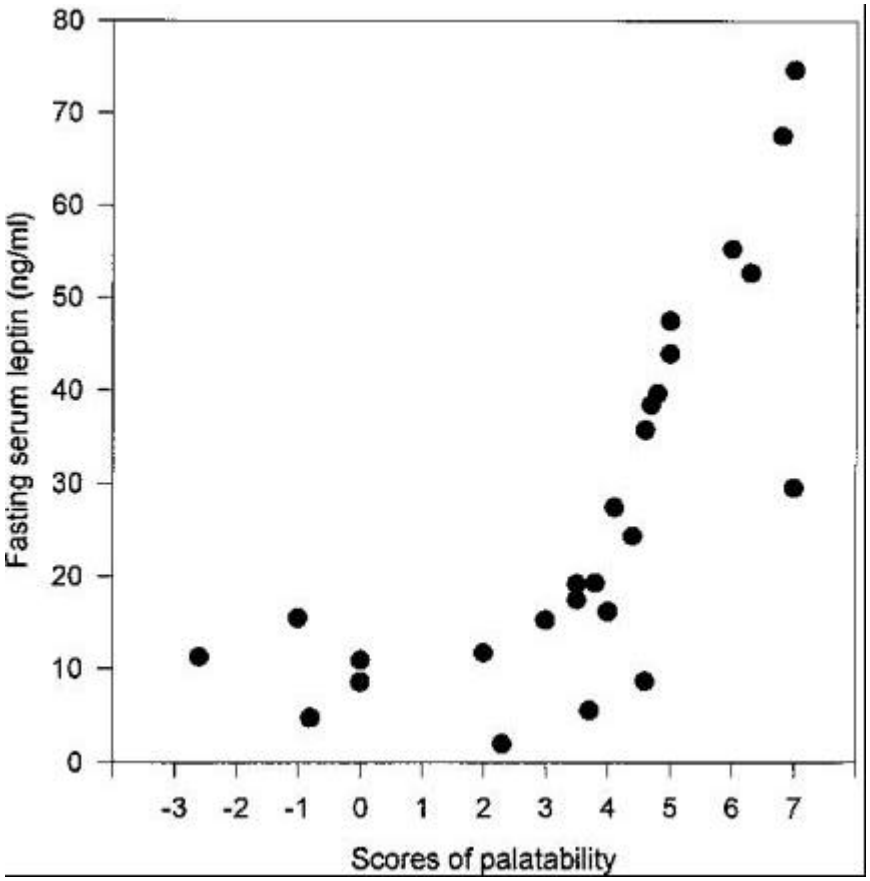

Figure 2 Correlation between fasting serum leptin and scores of palatability as assessed by a visual analogue scale

As far as we are aware, this is the first study reporting a positive correlation between the leptin system and palatability, as assessed psychometrically. This correlation remained significant after adjustment for BMI, body fat mass and percentage of body fat, which are usually the most important correlates of leptin: it cannot be explained by a possible bias towards the upper range of these three variables, which were normally distributed. Recently, Karhunen et al. [10] raised the question of a putative role for leptin in the regulation of feeding behaviour, in a population of obese binge- and non-binge-eating women. They found that serum leptin levels were not associated with the feeling of hunger or the desire to eat, as measured by visual analogue scales. Thus they concluded that leptin did not regulate the short-term appetite or satiety processes. In contrast to the above, our results suggest that leptin could mediate some aspects of eating behaviour: subjects with high basal leptin concentrations tend to exhibit increased perception of palatability, which leads to a number of intriguing questions. Theoretically, an inappropriate elevation of leptin would decrease appetite and food intake, and enhance thermogenesis. Moreover, high fasting leptin levels were associated with a decreased salivation response in the presence of food and food-related stimuli [10]. Nevertheless, it should be remembered that most obese subjects are leptin insensitive, which may explain in part our results.

In this study, we failed to observe an influence of palatability on insulin release, as reported in normal weight subjects by LeBlanc and Brondel [16]. Other studies confirmed that the effect of palatability on the magnitude of cephalic-phase responses, in particular diet-induced thermogenesis, was dependent on obesity status, being seen only in the non-obese [25]. We did not find that serum leptin levels were associated with hyperinsulinaemia or insulin resistance, independent of BMI or body composition. In addition, the stepwise regression analysis showed that leptin was the major statistical determinant of palatability. It can be speculated that the leptin system is acting at least indirectly on the perception of palatability and thus takes part in the control of food intake through this particular aspect of human behaviour. Alternatively, since this is a cross-sectional study, the direction of causality has to be further elucidated. It cannot be excluded that a state of leptin resistance and enhanced perception of palatability are concomitant expressions of the same dysregulation, leading to a decreased thermogenesis in overweight subjects. These hypotheses need to be investigated in a more extended population, in relation to weight status and gender.

In conclusion, although it seems paradoxical to find a positive correlation between fasting leptin and the perceived palatability, our results support the concept of a close relationship between leptin and eating behaviour in humans.

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