CLINICAL STUDY

No acute response of leptin to an oral fat load in obese patients and during circadian rhythm in healthy controls

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Abstract

This study was done to elucidate the relationship between postprandial leptin and obesity, and the possible influence of the circadian rhythm on the dynamic leptin response to an oral fat load (OFLT). In *experiment 1*, we measured the leptin and insulin responses to an oral fat load in 16 non-diabetic obese subjects and in 16 healthy controls, matched for age and gender. In *experiment 2*, we measured the leptin and insulin responses to an OFLT according to the time of fat load ingestion: 0700 h (diurnal (D) test) or 2200 h (nocturnal (N) test) in nine normal-weight healthy males. Baseline leptin concentration was correlated with the body mass index, body fat mass and percentage of body fat mass in both experiments. The leptin concentrations were higher in women than in men ($P < 0.001$). In *experiment 1*, the leptin concentrations were higher in obese subjects than in controls, but did not change over time in either group. The plasma insulin concentrations at baseline and during the postprandial state, as well as the area under the curve (AUC) of insulin, were higher in obese subjects than in controls ($P < 0.05 - 0.0001$). There was no correlation between postprandial insulin responses and postprandial leptin responses in either obese or control groups. In *experiment 2*, leptin (D vs N, 2.9 ± 1.4 vs 2.9 ± 1.0 ng/ml) and insulin (D vs N, 41 ± 18 vs 25 ± 9 pmol/l) concentrations were similar at the beginning of the D and N tests after a 10 h fast. The leptin concentrations did not change after D or N tests and were not statistically different for D and N tests. Our results indicate that the leptin concentration in healthy controls and in obese patients is not acutely influenced by a high fat load.

European Journal of Endocrinology 143 649–655

Introduction

Obesity is particularly frequent in western countries, with a prevalence of 15% in men and 20% in women aged 35–64 years in Europe (1, 2). The consumption of high-fat diets leads to increased energy intake, weight gain and obesity in humans (3), but the mechanisms by which increases in dietary fat lead to weight gain are not well understood. Leptin, the product of the *ob* gene, has a major impact on obesity. Animal studies indicate that leptin is involved in the control of food intake (4–6). The influence of leptin on energy intake in humans is supported by data on leptin-deficient subjects (7). The plasma leptin concentration is also influenced by chronic food intake (8), but little information is available on acute leptin regulation by different meals. Recently, it was demonstrated that the leptin concentrations in healthy subjects dropped after an oral glucose tolerance

test, or 2 h after a mixed meal (9). High-fat meals reduce 24 h circulating leptin concentrations more than do low-fat meals in healthy women (10), suggesting that leptin plays a role in the development of obesity induced by fat consumption. But no information is available on obese patients, and the acute relationship between obesity and the postprandial leptin response to an oral fatty meal remains to be determined.

Leptin secretion follows a circadian rhythm (11), with circulating leptin concentrations being high around midnight and low in the morning (12). These variations are linked to the pattern of meal eating, as suggested by meal shift experiments. The nocturnal leptin concentration has been negatively correlated with the risk to gaining weight (13), making the dynamic response to leptin an attractive field of research for factors influencing obesity. But no information is available on the effect of identical meals given

for breakfast (morning) or for dinner (evening), when subjects fasted for similar times before the test meal.

We performed two studies on the leptin responses to an oral fat load. The first examined the postprandial leptin responses of obese patients and normal-weight controls to an oral fat load. The second examined the postprandial leptin responses of normal-weight subjects to oral fat loads given at different times (diurnal at 0700 h or nocturnal at 2200 h).

Subjects and methods

Subjects

Experiment 1 We studied the 8 h postprandial leptin response to an oral fat load in 16 obese patients (4 men, 12 women) and 16 normal-weight controls (4 men, 12 women) recruited in the Clinical Research Center, Nancy (France). They were all normal on physical examination, had normal glucose tolerance (fasting plasma glucose < 6.1 mmol/l and 2 h plasma glucose < 7.8 mmol/l assessed by a 75 g oral glucose tolerance test) and no dyslipidemia in fasting blood sample (LDL cholesterol < 4.13 mmol/l, triglyceride < 1.70 mmol/l, and HDL cholesterol > 0.90 mmol/l for men or > 1.03 mmol/l for women). Patients and controls were matched for gender and age (± 3 years). All the women were premenopausal, with normal menstrual cycles and no subject was taking any medication.

Experiment 2 The postprandial leptin response of nine healthy male volunteers was studied for 8 h after an oral fat load administered at 0700 h (diurnal test, D) or at 2200 h (nocturnal test, N). All subjects underwent two consecutive oral fat load tests. Each subject served as his own control. The time between the two tests was 14 ± 1 days. Nocturnal and diurnal tests were performed in a random order. Five subjects started with the D test and four with the N test.

Body mass index (BMI) was used as an index of adiposity. Obesity was defined by a BMI above 30 kg/m^2 , and normal-weight by a BMI of $18\text{--}24.9 \text{ kg/m}^2$. Body weight was stable for obese and control subjects (less than 2% change in the previous 3 months). Abdominal (android) fat distribution was defined by a waist-to-hip ratio (WHR) of over 0.85 for women, and over 0.95 for men. Body composition was determined by dual-energy X-ray absorptiometry (DEXA) with a Lunar DPX-L machine (Lunar, Madison, WI, USA).

This project was approved by the local ethics committee of the Nancy University Hospital (France) and all subjects gave their written informed consent.

Oral fat load test (OFLT)

In both experiments, subjects spent at least 12 h in our Clinical Research Center before beginning the OFLT. They were instructed to refrain from strenuous exercise and

alcohol for 3 days prior to the OFLT. The subjects were given a calibrated meal corresponding to step I of the National Cholesterol Education Program (14) (690 Cal with 31% fat, 19% protein and 50% carbohydrate) at 2030 h on the day before the diurnal fat load. They fasted for 10 h, until 0800 h, at which time the diurnal oral fat load was given. In *experiment 2*, subjects were given the same calibrated meal at 2030 h on the day before the diurnal test and at 1130 h on the morning before taking the nocturnal fat load. They fasted for 10 h, until 2200 h, when the nocturnal fat load was given.

The fat load test was performed 2 weeks after the inclusion oral glucose challenge. The fat load was 180 g of a manufactured emulsified blended meal composed of 3.5% dried skimmed milk, 19.25% butter, 23.75% peanut oil, 22% chocolate, 30.25% water, 0.75% gelatin, 0.25% sorbic acid and 0.25% potassium sorbate (Laboratoire Pierre Fabre Santé, Castres, France). Its energy content was (15): 890 Cal (85% fat, 13% carbohydrates, 2% protein), with 35 g saturated fatty acid, 30 g mono-unsaturated fatty acid, 15 g poly-unsaturated fatty acid and 88 mg cholesterol. The fat load was ingested over 15 min., with 200 ml water. No further food or drink were allowed during the study. The participants remained supine and slept normally for the whole nocturnal test. They were instructed to remain in bed, supine, for the diurnal test.

Biochemical measurements

A 21 gauge indwelling venous canula was inserted into an antecubital vein 30 min prior to the fat load. Blood samples (15 ml) were then taken 30, 20 and 10 min before the fat load, to determine baseline insulin and glucose concentrations. The mean of the three values was considered. Blood samples were then taken immediately before the fat load and 2, 4, 6 and 8 h later (here designated t_0 , t_2 , t_4 , t_6 , t_8) to determine leptin and insulin concentrations. All blood samples were collected in vacutainer tubes and immediately centrifuged at 1000 g for 15 min, at 4°C . The tubes for measuring triglyceride in plasma contained a final concentration of 0.15% EDTA.

Total cholesterol and triglyceride concentrations were measured enzymatically (bioMérieux, Marcy l'Etoile, France). HDL cholesterol was assessed by phosphotungstic acid precipitation and LDL cholesterol was calculated according to the Friedewald formula (16). Plasma glucose was determined enzymatically (bioMérieux, PAP 250, Marcy l'Etoile, France). Total plasma insulin concentration was determined by immuno-enzymatic assay (Insulin IMX®, Abbott Laboratories, Tokyo, Japan). Cross-reactivity with pro-insulin was below 0.05%. Fasting plasma leptin concentrations were measured in triplicate by radio-immunoassay kit (LINCO Research Inc., St Louis, MO, USA). The intra- and interassay coefficients of variation were 4.5 and 8%, respectively.

Table 1 Clinical characteristics of the obese patients and controls at inclusion.

	Experiment 1			Experiment 2
	Obese (<i>n</i> = 16)	Controls (<i>n</i> = 16)	<i>P</i> value*	Controls (<i>n</i> = 9)
Age (years)	40±7	38±7	NS	22±3
Sex (male/female)	4/12	4/12	NS	9/0
BMI (kg/m ²)	34.4±3.2	21.5±2.2	<0.0001	22.4±0.9
Fat mass (kg)	38.8±6.3	18.6±3.1	<0.001	9.6±2.7
Fat mass (%)	41.7±5.2	28.7±4.6	<0.01	14.0±4.0
Waist circumference (cm)	108±9	73±9	<0.0001	77±3
Waist/hip ratio	0.94±0.06	0.80±0.09	<0.001	0.83±0.03

*Student's unpaired *t*-test between obese and control subjects for experiment 1.
NS, not significant.

Insulin sensitivity was assessed from blood samples collected 30, 20 and 10 min before the ingestion of the OFLT using the homeostasis model assessment (HOMA) system described by Matthews *et al.* (17) with the formula: ([insulin] × plasma [glucose])/22.5, where [insulin] is expressed in mU/l and plasma [glucose] in mmol/l.

Statistical analysis

Data are presented as means ± s.d. When the distribution of a variable was not normal, as assessed by skewness and kurtosis tests, data were log-transformed before statistical analysis was performed. Area under the time-dependent concentration curve (area under curves, AUC) was calculated by the trapezoidal rule (18). Two-way repeated-measures ANOVA was used to assess the effect of postprandial times on postprandial lipids, leptin and insulin concentrations. ANOVA was used with sample time and obesity status (*experiment 1*) or diurnal/nocturnal test (*experiment 2*) as factors affecting lipids, leptin or insulin concentrations. When ANOVA was significant, or when variables were measured only once, means were compared using Student's unpaired (*experiment 1*) or paired (*experiment 2*) *t*-test. The association between two continuous variables was determined by the linear regression coefficient. The level of significance was implied at *P* < 0.05. StatView® software (Abacus Concepts Inc.,

StatView V; Brain Power, Calabasas, CA, USA) was used for all calculations. To better clarify the illustrations, the data in the figures are shown as means ± s.e.m.

Results

The clinical characteristics at inclusion and the laboratory data at baseline of the subjects are summarized in Tables 1 and 2.

Experiment 1

Sexual dimorphism was evidenced by two-factor ANOVA (*F* = 17.24, *P* = 0.001), independent of the weight status (interaction between weight status and leptin at *t*0: *F* = 4.91, *P* = 0.05). Obese and control women had significantly higher leptin values (29.6 ± 9.9 and 9.0 ± 4.8 ng/ml, respectively) than obese and control men (11.2 ± 4.6 and 3.4 ± 1.5 ng/ml, respectively) (*P* < 0.01 and 0.05, respectively). However, the ratio of fasting leptin to percentage of body fat mass was not statistically different between obese subjects (0.58 ± 0.25) and controls (0.34 ± 0.16, *P* = 0.21).

A positive relationship was found between baseline leptin concentration (*t*0) and BMI (*r* = 0.72, *P* < 0.0001) and percentage of fat mass (*r* = 0.69, *P* < 0.01). The correlation between leptin at *t*0 and the body fat mass was not statistically significant (*r* = 0.48,

Table 2 Baseline laboratory characteristics of the obese patients and controls in experiments 1 and 2.

	Experiment 1			Experiment 2
	Obese (<i>n</i> = 16)	Controls (<i>n</i> = 16)	<i>P</i> value*	Controls (<i>n</i> = 9)
Leptin (ng/ml)	25.0±12.0	7.6±4.9	<0.0001	4.7±3.1
Leptin/% fat mass ratio	0.58±0.25	0.34±0.16	NS	0.31±0.12
HOMA	2.72±1.18	0.95±0.37	<0.0001	1.34±0.45
Total cholesterol (mmol/l)	4.68±0.72	4.79±0.72	NS	4.05±0.79
Triglyceride (mmol/l)	0.93±0.23	0.79±0.37	NS	0.83±0.28
HDL-cholesterol (mmol/l)	1.10±0.27	1.34±0.25	<0.05	1.16±0.21
LDL-cholesterol (mmol/l)	3.16±0.61	3.09±0.58	NS	2.51±0.73

*Student's unpaired *t*-test between obese and control subjects for experiment 1.

Figure 1 Experiment 1: plasma postprandial triglyceride (upper panel), leptin (middle panel) and insulin (lower panel) concentrations for 16 obese patients (●) and 16 controls (○) at baseline and for 8 h after ingestion of the test meal at t_0 . Values are shown as means $\pm$ S.E.M. * $P < 0.05$; obese patients vs controls; $P < 0.05$ vs value at t_0 in obese patients. Means were compared using Student's unpaired t -test.

$P = 0.052$). HOMA was also well correlated with the leptin levels at baseline ($r = 0.68$, $P < 0.001$).

The triglyceride (TG) response to the OFLT is illustrated in Fig. 1 (upper panel). The total areas under the plasma triglyceride curve were similar in the

Figure 2 Experiment 2: plasma postprandial triglyceride (upper panel) and leptin (lower panel) responses in 9 healthy men at baseline and for 8 h after diurnal (□) and nocturnal (■) oral fat load tests. Values are shown as means $\pm$ S.E.M. Means were compared using Student's paired t -test.

obese (696 ± 178 mmol/(l min)) and control groups (684 ± 250 mmol/(l min)). The plasma TG concentrations tended to return to initial values in the control group after 8 h (0.92 ± 0.40 vs 0.79 ± 0.37 mmol/l, $P = 0.29$), whereas they remained elevated in the obese group (1.24 ± 0.46 vs 0.93 ± 0.23 mmol/l, $P < 0.01$) and significantly higher than in controls ($P < 0.05$).

The leptin and insulin responses to the OFLT are illustrated in Fig. 1 (middle and lower panels, respectively). The baseline leptin concentration in obese subjects (25.0 ± 12.0 ng/ml) was higher than in controls (7.6 ± 4.9 ng/ml, $P < 0.0001$). The leptin concentrations did not change significantly over the postprandial time ($F = 1.47$, $P = 0.22$), and the interaction between sample time and obesity status was not significant ($F = 1.29$; $P = 0.28$), indicating that the postprandial leptin responses of obese patients and controls did not differ. Only the leptin concentration at t_6 (22.5 ± 10.3 ng/ml) was lower than the fasting value in obese subjects (25.0 ± 12.0 ng/ml, $P < 0.05$).

The postprandial insulin response was strongly influenced by obesity ($F = 38.51$; $P < 0.0001$) and the postprandial sample time ($F = 16.77$, $P < 0.0001$), but the interaction was not statistically significant ($F = 1.58$, $P = 0.18$), indicating that the postprandial insulin response curves were parallel and did not differ significantly for obese and controls. However, as expected, baseline insulin levels were significantly higher in obese patients (78 ± 26 pmol/l) than in controls (34 ± 13 pmol/l, $P < 0.05$), as well as the AUCs of insulin ($41\,969 \pm 15\,295$ vs $18\,242 \pm 5081$ pmol/l min), $P < 0.0001$). The AUC of insulin was not significantly correlated with AUC of leptin in either obese ($r = 0.34$; $P = 0.20$) or in control subjects ($r = 0.23$; $P = 0.41$).

Experiment 2

A positive correlation was found between baseline diurnal leptin (t_0) and BMI ($r = 0.76$, $P = 0.05$), body fat mass ($r = 0.85$, $P = 0.01$) and percentage of body fat mass ($r = 0.91$, $P = 0.001$). A similar trend was observed with baseline nocturnal leptin, but the correlation was not significant ($r = 0.68$, $P = 0.06$; $r = 0.69$, $P = 0.057$, for body fat mass and percentage of body fat mass, respectively). Therefore, HOMA was correlated with leptin levels at baseline, only in the morning ($r = 0.72$, $P = 0.02$), but not significantly in the night.

The total AUC for plasma triglyceride from the diurnal and nocturnal tests were similar. Two-way repeated-measures ANOVA showed a significant effect of the interaction between the time of the fat load test administration and postprandial times on triglyceride concentrations ($F = 5.03$, $P < 0.0001$), indicating that the curves for plasma triglyceride concentrations after each test were significantly different (Fig. 2, upper panel). The triglyceride concentrations at t_2 were higher in the diurnal test (1.51 ± 0.69 mmol/l) than in the nocturnal test (1.04 ± 0.58 mmol/l, $P < 0.05$). But the triglyceride values at t_8 were higher in the nocturnal test (1.52 ± 0.63 mmol/l) than in the diurnal test (0.82 ± 0.34 mmol/l, $P < 0.05$).

The leptin responses to diurnal and nocturnal fat loads are illustrated in Fig. 2 (lower panel). Baseline leptin concentrations at 0700 h (2.9 ± 1.4 ng/ml) and 2200 h (2.9 ± 1.0 ng/ml, $P = 0.58$) were not different. The leptin concentrations did not change significantly over the postprandial time ($F = 0.78$; $P = 0.55$), and the interaction between sample time and the time of fat load test administration (diurnal vs nocturnal) was not significant ($F = 0.63$; $P = 0.64$). The postprandial leptin concentrations were not different from the baseline leptin value, after either the diurnal or the nocturnal test.

The baseline insulin concentrations at 0700 h (41 ± 18 pmol/l) and 2200 h (25 ± 9 pmol/l, $P = 0.28$) were not different. The curves for plasma insulin

concentrations after each test were similar (data not shown).

Discussion

Our results show that there is no acute leptin response in the 8 h following an oral fat load in obese or control subjects, and that there appears to be no acute leptin change in normal-weight controls after a fat load test administered at 0700 h or at 2200 h. We also found no relationship between the postprandial insulin response and the leptin concentration. Our results for anthropometric characteristics are in good agreement with previous reports; we find a sexual dimorphism in the fasting leptin concentration, with higher values in women (19). We also find a correlation between fasting leptin and adiposity, as generally reported (20, 21). These results were found in obese as well as in healthy controls, but not significantly in healthy controls in the night.

Leptin, produced by adipose tissue, regulates adipose tissue mass, and circulating leptin levels are directly proportional to total adipose mass. Leptin concentration is also sensitive to caloric balance and influenced by chronic food intake: restriction of food intake leads to a drop in circulating leptin greater than that predicted by the reduction in adipose mass (8).

But there is little information available on acute leptin regulation by different meals. Our present findings indicate a lack of acute postprandial regulation of leptin by fat intake. This agrees with several studies (11, 21, 22). Some studies have shown that leptin levels do not increase after meals (21) or a glucose load (23). However, a recent study found that leptin concentrations dropped by $8.8 \pm 4.4\%$ (9), confirming the study of Mohamed-Ali *et al.* (24), who showed that leptin levels remained unchanged 1 and 2 h after a high-carbohydrate meal, but increased after 3–5 h.

The effect of fat on leptin secretion is not well understood, particularly in obese patients, and the acute relationship between obesity and the postprandial leptin response to fat intake remains to be determined. In a recent study, Havel *et al.* showed that the postprandial leptin response of normal-weight women to a high fat meal reduces the nycthemeral leptin response more than a high-carbohydrate meal (10). This decrease in 24 h circulating leptin could contribute to the weight gain produced by the consumption of high-fat diets. Some authors have found no postprandial leptin response 3 h after a meal in healthy controls or obese patients (25). Our study was conducted over 8 h, and a high fat test was used (fat represented 85% of the caloric intake, which is best suited to discriminating the metabolic changes specifically due to the acute fat intake without interference from the effect of carbohydrate). The acute circulating leptin response to such a fatty meal has never been studied, particularly in obese subjects. We failed to demonstrate an acute regulation of leptin by fat intake

in obese subjects. A reason for the decrease in leptin concentration 6 h after the fat load in obese subjects is unclear. Although of small amplitude (about 10%), this postprandial decrease in leptin level 6 h after a fatty meal has not been previously reported, but could correspond to a decrease in leptin at 10.00 am in obese subjects (9, 12). Our results must be linked to the finding that changing the fat content of the diet has no effect on fasting leptin after a period of 7 days (20).

Two studies suggest that insulin is the signal that mediates the effect of caloric intake on leptin production (9, 24). In *experiment 1*, we find that the mean increase in insulin concentration after a fat load is modest in obese subjects (at t_2 107 ± 34 pmol/l) as well as in controls (at t_2 49 ± 29 pmol/l), well below the increases that induce a leptin response (26, 27). Saad *et al.* showed that the increase in leptin concentration during insulin infusion is 50% of the reference point for a plasma insulin concentration of 138 ± 36 pmol/l (28). The change in leptin concentration was inversely related to plasma insulin concentration. It has also been demonstrated that leptin concentrations are increased only 6 h after an insulin infusion in normal subjects, and after 8.5 h in patients with type 2 diabetes mellitus (26). The lack of correlation in our study between insulin and leptin responses supports the lack of an acute regulation of leptin by insulin in physiological situations.

In *experiment 2*, the lack of difference in baseline leptin concentration between 0700 h and 2200 h is in apparent disagreement with those studies showing a peak in leptin concentration around midnight and a nadir around 10.00 h (12, 22, 29, 30). There is a nocturnal increase in leptin, however, which is related to the insulin response to meals. Leptin levels usually fall in the morning. But we gave a high-fat meal at the end of the day, after a fast similar to that experience at night to assess the effect of the meal itself on plasma leptin, independent of circadian changes in hormone secretion, i.e. cortisol levels. Consequently, we observed similar baseline levels of leptin whether in the morning or at night. This result is in agreement with the study of Schoeller *et al.* showing that a meal shift results in a shift in the leptin rise (12). In that study, the authors concluded that leptin rhythmicity was related to meal timing rather than light-dark cycle. The fast undergone by our subjects between 12.00 h and 2200 h was equivalent to a meal shift, and, the normal cumulative effect of the meals on the late evening leptin levels was thus lost. As the meal-related cumulative rise in insulin concentration is known to be the main factor determining the nocturnal increase in leptin (19), the lack of difference between diurnal and nocturnal basal leptin is not unexpected, as baseline insulin concentrations in the diurnal and nocturnal tests were not different. The nocturnal rise in leptin observed in other studies could be due only to the cumulative effect of the meals during the day (10, 11). Although Dallongeville *et al.* used a different test meal,

they also failed to find any difference between the postprandial response at different times of day (31).

The fact that the correlations between leptin and adiposity or HOMA observed for the diurnal test disappeared for the nocturnal test, suggests that other mechanisms, like the leptin pulse amplitude (32), could be involved in the regulation of leptin secretion during the day. The magnitude of the relative diurnal change in leptin has also been reported to be higher in lean men than in other lean or obese populations (19). In our small population of healthy men, we cannot exclude the influence of these regulations.

Our results thus provide evidence that fat intake is not linked to an acute change in postprandial leptin, and that the leptin concentration is not acutely influenced by an oral fat load. The lack of any relationship between obesity and the leptin response to an acute fat load makes it imperative to investigate further the regulatory process linking dietary fat intake and the development of obesity.

Acknowledgements

This work was supported by a grant from the Ministère de la Santé et de la Solidarité Nationale: Projet Hospitalier de Recherche Clinique. Experimental oral fat loads were supplied by Laboratoires Pierre Fabre Santé, Castres, France. We thank the staff of the Centre d'Investigation Clinique du CHU de Nancy for their clinical and technical help. We thank Dr Owen Parkes for editing the English in this manuscript.

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Received 04 December 1999

Accepted 29 June 2000