High-Fat Meals Reduce 24-h Circulating Leptin Concentrations in Women

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Leptin induces weight loss in rodents via its effects on food intake and energy expenditure. High-fat diets induce weight gain, but the mechanism is not well understood. Previous studies have not found an effect of dietary fat content on fasting leptin. There is a nocturnal increase of leptin, however, which is related to insulin responses to meals. We have reported that adipocyte glucose utilization is involved in insulin-induced leptin secretion in vitro. Accordingly, high-fat, low-carbohydrate (HF/LC) meals, which induce smaller insulin and glucose responses, would produce lower leptin concentrations than low-fat, high-carbohydrate (LF/HC) meals. Blood samples were collected every 30–60 min for 24 h from 19 normal-weight (BMI, 24.2 ± 0.7 kg/m^2; percent body fat = 31 ± 1%) women on 2 days (10 days apart) during which the subjects were randomized to consume three isocaloric 730-kcal meals containing either 60/20 or 20/60% of energy as fat/carbohydrate. Overall insulin and glycemic responses (24-h area under the curve [AUC]) were reduced by 55 and 61%, respectively, on the HF/LC day ($P < 0.0001$). During LF/HC feeding, there were larger increases of leptin 4–6 h after breakfast (38 ± 7%, $P < 0.001$) and lunch (78 ± 14%, $P < 0.001$) than after HF/LC meals (both $P < 0.02$). During LF/HC feeding, leptin increased from a morning baseline of 10.7 ± 1.6 ng/ml to a nocturnal peak of 21.3 ± 1.3 ng/ml (change, 10.6 ± 1.3 ng/ml; percent change, 123 ± 16%; $P < 0.0001$). The amplitudes of the nocturnal rise of leptin and the 24-h leptin AUC were 21 ± 8% ($P < 0.005$) and 38 ± 12% ($P < 0.0025$) larger, respectively, on the LF/HC day. In summary, consumption of HF/LC meals results in lowered 24-h circulating leptin concentrations. This result may be a consequence of decreased adipocyte glucose metabolism. Decreases of 24-h circulating leptin could contribute to the weight gain during consumption of high-fat diets. *Diabetes* 48:334–341, 1999

Consumption of high-fat diets leads to increased energy intake, weight gain, and obesity in humans (1–3) and animals (4–6). Ready availability of high-fat foods is likely to contribute to the high prevalence of obesity in Western countries (7,8) and in developing countries in which traditional diets are replaced by diets with a higher fat content (3). The mechanisms by which increases of dietary fat lead to weight gain are poorly understood. Several studies have suggested that leptin is unlikely to have a role in the development of obesity induced by fat consumption, since short-term variations in dietary fat content (9,10), or long-term variations when body weight was unchanged (11), did not alter fasting leptin concentrations. Because circulating leptin concentrations exhibit a diurnal pattern, with peak nocturnal concentrations up to two times higher than nadir levels (12–14), we hypothesized that it might be necessary to examine leptin concentrations over a 24-h period to observe an effect of dietary macronutrient content. Thus, previous studies that examined only fasting morning plasma leptin levels may have missed an effect of dietary fat content to alter leptin concentrations at other times.

Meals that are high in fat content induce smaller excursions of circulating insulin and glucose (15). Although plasma leptin concentrations are highly correlated with adipose mass in humans (10,11,16,17) and animals (5), adiposity is not the sole determinant of circulating leptin. For example, circulating leptin concentrations decrease out of proportion with the relatively modest changes of body weight and adiposity during short-term fasting (9,18,19) or energy restriction (20) and are restored after refeeding (9,21). Several studies have suggested that changes of insulin and glucose are involved in regulating these changes of circulating leptin concentrations. For example, changes of leptin during 7 days of energy restriction are related to changes of glycemia (20). Moreover, the decline of leptin during fasting can be prevented or reversed by infusing modest amounts of glucose to prevent or reverse the concurrent decline of plasma glucose and insulin concentrations (18,19). The nocturnal rise of leptin is related to insulin responses to meals (13,14) and does not occur if the subjects are fasted (18). Finally, the timing of the peak and nadir of plasma leptin can be reset forward by 5–7 h by delaying the timing of meal feeding by 6.5 h (22).

We have recently reported that insulin increases leptin expression and secretion from isolated adipocytes via its actions to increase adipocyte glucose uptake and metabolism (23). These data suggest that the effects of fasting and refeeding on circulating leptin in vivo may be due to insulin- and glucose-mediated changes of adipose tissue glucose utilization. If circulating insulin and glucose influence leptin secretion, then it might be expected that low-fat, high-carbohydrate (LF/HC) meals, which produce greater insulin secretion and larger postprandial glycemic excursions, would result in higher plasma leptin concentrations over a 24-h period than high-fat, low-carbohydrate (HF/LC) meals. Since there is evidence that leptin is involved in regulating adiposity in humans (24,25), decreased 24-h leptin profiles could

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AUC, area under the curve; GCRC, General Clinical Research Center; HF/LC, high-fat, low-carbohydrate; HPA, hypothalamic-pituitary-adrenal; LF/HC, low-fat, high-carbohydrate.

contribute to weight gain in people consuming diets that are high in fat.

We therefore collected blood samples for leptin measurement from women at 30- to 60-min intervals for a 24-h period during which the subjects consumed three meals with either HF/LC or LF/HC content. We assessed the effect of altering dietary macronutrient content on insulin and glycemic responses to meals and 24-h circulating leptin concentrations. Because plasma cortisol concentrations have been implicated in the regulation of 24-h circulating leptin profiles (13,26,27), we also examined plasma cortisol profiles.

RESEARCH DESIGN AND METHODS

Subjects. The experimental protocols were approved by the Committee on Studies Involving Human Subjects at the University of Pennsylvania, and each subject gave informed written consent for the procedures. Nineteen women were studied. They averaged 26.4 ± 1.5 years of age (range 20–43) with a mean BMI of 24.2 ± 0.7 kg/m^2 (range 20.0–29.5), a percent body fat of 30.5 ± 1.4% (range 20–41), and a total fat mass of 20.1 ± 1.5 kg (range 12.5–35.3). Fasting hormone and substrate levels for the subjects on the two study days are shown in Table 2. Subjects underwent a telephone interview to assess eligibility, followed by a physical examination which included an electrocardiogram and a medical history to ensure that they had no chronic illnesses, abnormal heart rhythms, hypertension, or family history of diabetes or hypertension. A blood sample was drawn after an overnight fast. Subjects whose fasting blood glucose was >90 mg/dl, blood pressure >140/90 mmHg, or hemoglobin levels <11 g/dl were excluded from the study. Percent body fat was determined in each subject by bioelectrical impedance (model #BIA-101A; RJL Systems, Mt. Clemens, MI).

Experimental protocol. On the evenings before the experimental days, subjects entered the General Clinical Research Center (GCRC) at the Hospital of the University of Pennsylvania at 1700. Subjects were given dinner at 1800 and a snack at 2000 after which they fasted until the following morning. Subjects were permitted to select the type and quantity of food ingested at dinner; the snack ranged from 150 to 200 kcal. At 0730 the next morning, an intravenous catheter was inserted and kept patent with saline. Blood sampling commenced at 0800 and continued until 0800 the following morning (see blood sampling times below). Subjects were discharged from the GCRC upon completion of the blood sample collections.

Two experimental protocols were conducted (n = 9 and n = 10). The experimental protocols for the two groups of subjects differed only in the types of foods used in the meals; the macronutrient and energy content of the LF/HC meals and the HF/LC meals in the two groups of subjects were identical (Table 1). The purpose of changing the meals in the second group of 10 subjects was to confirm that the effects observed in the first 9 subjects were independent of any specific food item. Since there were no differences in the responses between the two groups of subjects (i.e., no effect of specific types of foods), the two groups were combined. Thus, each subject (n = 19) was studied on 2 experimental days on which either a LF/HC diet or a HF/LC diet was consumed. The two diets were administered to the subjects in a random order with a minimum 10-day interval between experimental days. Subjects were instructed to maintain their normal dietary intake and level of exercise during the 10-day interval. On one day, subjects ingested LF/HC meals, while on the other day, they ingested HF/LC meals. The meals in the two protocols differed only in the macronutrient composition, but not in energy content. On the LF/HC day, subjects ingested three identical meals containing 20% fat, 60% carbohydrate, and 20% protein; on the HF/LC day, subjects ingested three identical meals containing 60% fat, 20% carbohydrate, and 20% protein. Each meal, whether high in carbohydrate or fat, consisted of typically consumed whole foods and contained 733 kcal for a total of 2,200 kcal per day (Table 1). The HF/LC diets contained 4.2 g of dietary fiber, and the LF/HC diets contained 7.1 g of dietary fiber. Breakfast was given at 0900, lunch at 1300, and dinner at 1800. Subjects were required to ingest all of the food given and were not permitted any between-meal or after-dinner snacks.

Blood sampling. Blood samples were taken at 30-min intervals around periods of meal ingestion and during the predicted nocturnal rise of leptin secretion (5 h after the evening meal) or at hourly intervals at other times. After the baseline samples, which were collected at 0800, 0830, and 0900 before ingestion of the first meal, samples were taken at 0930, 1000, 1030, 1100, 1200, 1300, 1330, 1400, 1430, 1500, 1600, 1700, 1800, 1830, 1900, 1930, 2000, 2100, 2200, 2300, 2330, 2400, 0030, 0100, 0130, 0200, 0230, 0300, 0400, 0500, 0600, 0700, and 0800. Each sample collection involved the removal of 1 ml of blood to clear the catheter tubing, followed by a 5-ml collection into Vacutainer tubes containing EDTA. Aprotinin (Trasylol) and leupeptin were added, and the samples were kept on ice for not longer than 1 hour. Samples were centrifuged, separated into aliquots, and stored at –70°C until assayed.

Assays. Plasma insulin and glucagon were measured in duplicate using commercially available double-antibody radioimmunoassays purchased from Linco Research (St. Charles, MO). Radioimmunoassays for insulin and glucagon were performed by the Diabetes Research Center of the University of Pennsylvania. Technicians were blind to the conditions of the experiment. Plasma glucose and lactate were measured with a YSI 2300 StatPlus Glucometer (Yellow Springs Instruments, Yellow Springs, OH). Plasma leptin was measured with a radioimmunoassay for human leptin with reagents from Linco Research as previously described (28). Plasma cortisol was measured by radioimmunoassay with an Immuchem coated tube kit (ICN Biomedical, Costa Mesa, CA).

Data analysis. The area under the curve (AUC) was calculated for glucose, insulin, lactate, and glucagon using a computerized trapezoidal method (GraphPlot, Inplot, San Diego, CA). The mean of the three baseline values was determined, and net AUC was calculated by subtracting the areas below baseline from AUC values above baseline. AUCs for insulin, glucagon, glucose, and lactate are expressed as units per 23 hours, as the 1st hour of sampling was to determine baseline. The nadir and peaks for plasma cortisol were determined as the mean of the two lowest consecutive nighttime or two highest consecutive morning values, respectively. The nadirs and peaks for plasma leptin were determined as the two lowest consecutive morning values before 1200 and the two highest consecutive

TABLE 1
Composition of the diets

Food	Quantity (g)
LF/HC meals: 20% fat, 60% carbohydrate, 20% protein; 733 kcal	
Meal set 1 (9 subjects)	
Whole wheat bread	66
Turkey breast	94
Iceberg lettuce	15
Tomato	30
Nonfat skim milk	130
Orange juice	265
Banana	100
Vanilla pudding	100
American cheese	21
Mustard	10
Meal set 2 (10 subjects)	
White bread	66
Turkey breast	35
Egg yolk substitutes	63
Egg white	49
Cheddar cheese	20
Butter	8
Mashed potatoes (dry)	85
Water (for potatoes)	400
HF/LC meals: 60% fat, 20% carbohydrate, 20% protein; 733 kcal	
Meal set 1 (9 subjects)	
Whole wheat bread	57
Turkey breast	105
Iceberg lettuce	15
Tomato	30
Whole milk	190
Margarine	10
Canola oil	10
Mayonnaise	22
American cheese	23
Meal set 2 (10 subjects)	
Whole wheat bread	66
Turkey breast	49
Egg yolk	46
Egg white	25
Cheddar cheese	36
Butter	20
Half and half cream	30

TABLE 2
Baseline (fasting) insulin, glucagon, glucose, and lactate concentrations and AUCs above baseline 0800–0900 for the 23 h after the baseline samples were collected

	Baseline		AUCs over 23 h	
	LF/HC	HF/LC	LF/HC	HF/LC
Insulin (pmol/l)	42.7 ± 3.9	47.0 ± 3.7†	2,930.0 ± 338.0	1,307.3 ± 169.4*
Glucagon (ng/l)	46.0 ± 3.0	49.6 ± 3.2†	246.3 ± 48.2	258.0 ± 41.5†
Glucose (mmol/l)	3.9 ± 0.1	4.1 ± 0.1†	15.66 ± 1.72	6.04 ± 1.43*
Lactate (mmol/l)	0.7 ± 0.1	0.7 ± 0.1†	3.79 ± 1.01	–1.54 ± 0.89*

Data are means ± SE. *P < 0.0001; †NS vs. LF/HC.

nighttime values after 2000, respectively. Thus, the peaks and nadirs for cortisol and leptin did not occur at exactly the same time in each subject. This leads to an apparent blunting of the peaks and nadirs when the results are presented as a time-course in the figures compared with the mean data presented in the tables. The AUCs for leptin and cortisol are expressed as units above each subject's nadir over 5, 12, or 24 h. Differences between treatments over time were determined by ANOVA and examined for time × treatment interactions with a Tukey's post test. Differences between overall responses to the two diets were determined with paired t tests. Linear regression analysis was also performed. Results are presented as mean ± SE. Significance was defined as P < 0.05.

RESULTS

There were no significant differences in subject characteristics or in any of the physiological variables measured between the subjects eating the meal sets with different food items of the same macronutrient composition. Because similar effects of changing the macronutrient intake on plasma leptin levels were observed with both meal sets, the results from all 19 subjects were combined. Subjects maintained a consistent weight between the two test days. Mean weight for the 19 subjects was 65.0 ± 2.5 kg on the HF/LC day and 64.6 ± 2.5 kg on the LF/HC day (t = 0.66, P = 0.53). The total energy expenditure required for the women participating in the study was calculated to be 2,299 ± 118 based on an estimate of 35 kcal · kg^{-1} · day^{-1} (assuming a light activity level). A mean difference of –3 ± 4% was found between the estimated total energy requirements and the number of kilocalories consumed by the study participants. The difference between estimated total energy requirements and quantity of food consumed was not significantly different between subjects eating the two different meal sets (F = 0.09, P < 0.77).

Plasma insulin and glucagon responses. There were no significant differences in mean fasting insulin or glucagon concentrations between the two treatment days (Table 2). Peak insulin concentrations on the HF/LC diet were significantly lower after each meal compared with the LF/HC diet (%Δ at breakfast, –46 ± 7%, at lunch, –36 ± 11%, and at dinner, –48 ± 11%; all P < 0.0025), but the timing of the peaks was not significantly different (Fig. 1). The AUC for insulin on the HF/LC day was decreased by 53 ± 3% relative to the LF/HC day (P < 0.0001; Table 2). There were no significant differences in plasma glucagon profiles (Fig. 2) or the 23-h AUCs for glucagon between the two treatments (Table 2).

Plasma glucose and lactate responses. Baseline fasting glucose concentrations were similar on the two study days (Table 2). Plasma glucose concentrations after each meal were lower after HF/LC than after LF/HC meals (all P < 0.005; Fig. 3). The overall increment of plasma glucose concentrations over baseline concentrations as assessed by the AUC for glucose was decreased by 67 ± 11% (P < 0.0001) on the HF/LC day versus the LF/HC day (Table 2). Baseline plasma

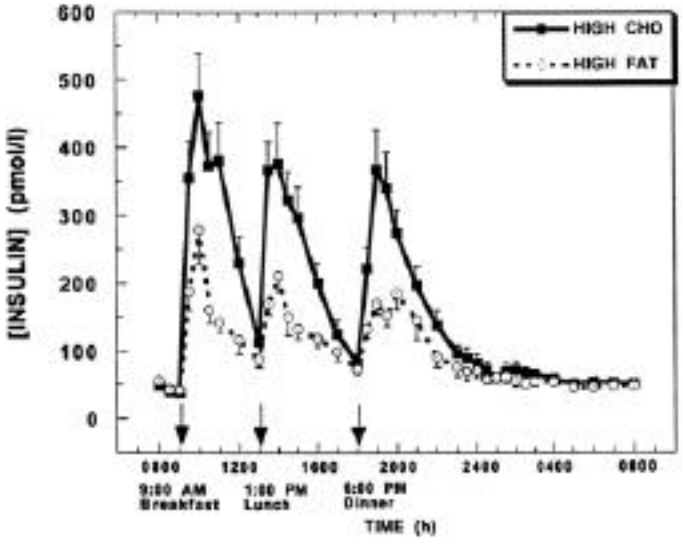

FIG. 1. Plasma insulin concentrations during a 24-h period (0800–0800) in 19 women consuming three LF/HC or three HF/LC meals.

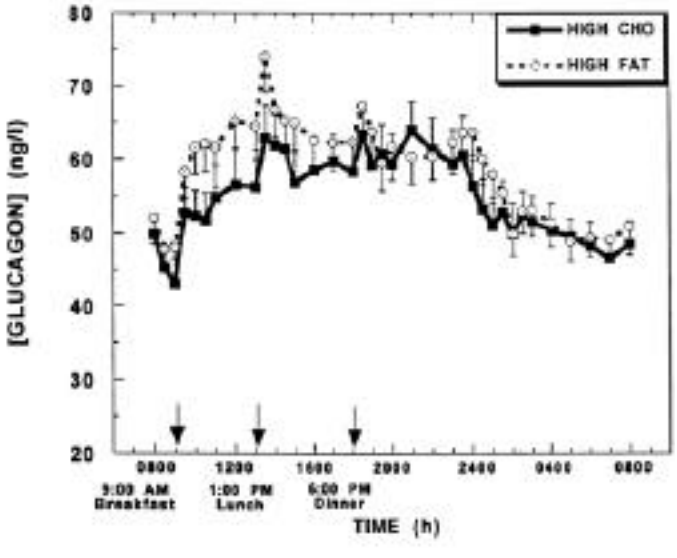

FIG. 2. Plasma glucagon concentrations during a 24-h period (0800–0800) in 19 women consuming three LF/HC or three HF/LC meals.

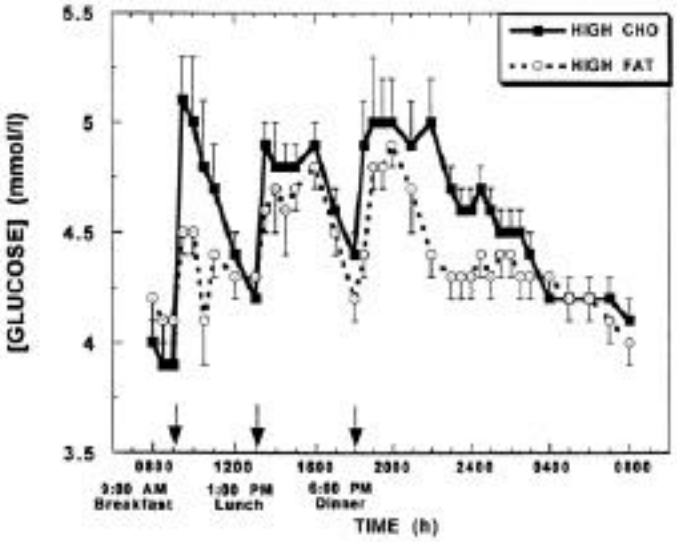

FIG. 3. Plasma glucose concentrations during a 24-h period (0800–0800) in 19 women consuming three LF/HC or three HF/LC meals.

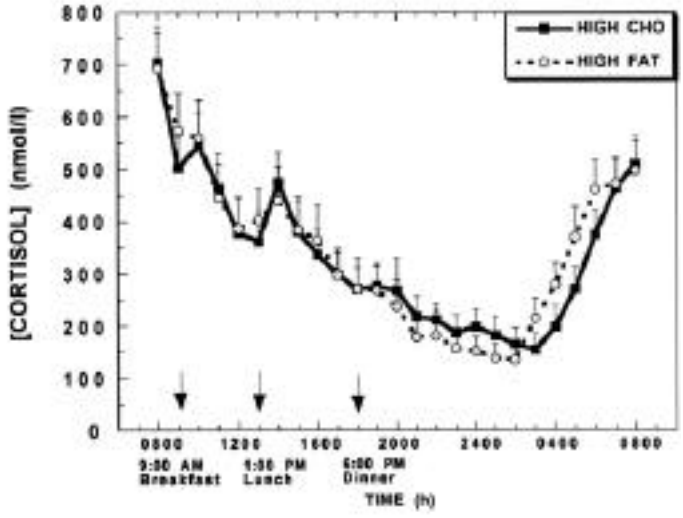

FIG. 5. Plasma cortisol concentrations during a 24-h period (0800–0800) in 19 women consuming three LF/HC or three HF/LC meals.

lactate concentrations were not different between the two treatment days (Table 2). Plasma lactate increased after each meal in a pattern similar to the insulin responses, and these increases were larger on the LF/HC than the HF/LC day (all $P < 0.0001$; Fig. 4). The average overall AUC above baseline for lactate was positive on the LF/HC day and negative on the HF/LC day (Table 2).

24-h plasma cortisol profiles. Plasma cortisol concentrations were highest at 0800 (Fig. 5). Peak and nadir cortisol concentrations as defined by the mean of two highest or lowest respective consecutive values were not different between the LF/HC and HF/LC study days. Similarly, the amplitude of the peak minus the nadir and the 24-h AUC over nadir levels were not different between the two diets (Table 3); however, the time of the early-morning nadir was delayed by ~1.5 h on the LF/HC day, occurring at 1,134 ± 131 min after the start of blood sampling (0800) vs. at 1,036 ± 98 min on the HF/LC day ($t = 2.87$, $P = 0.010$) (Fig. 5).

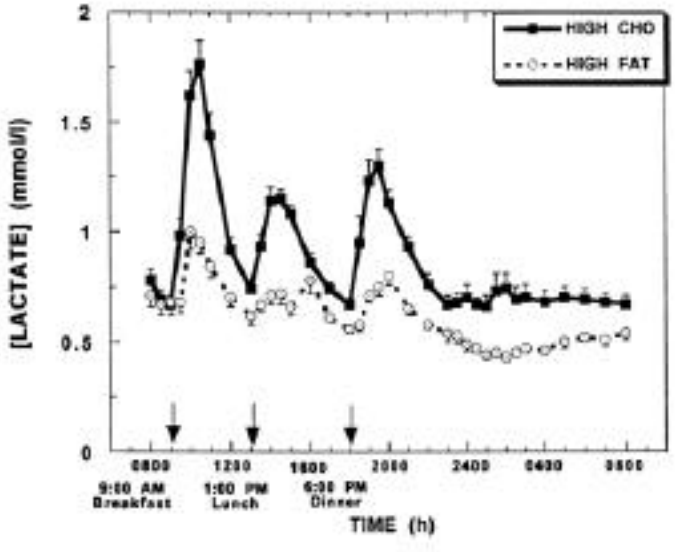

FIG. 4. Plasma lactate concentrations during a 24-h period (0800–0800) in 19 women consuming three LF/HC or three HF/LC meals.

Correlations between leptin and adiposity. Adiposity as assessed by BMI, percent body fat, or total body fat (fat mass) was significantly correlated with 0800 plasma leptin concentration, nadir and peak concentrations, amplitude of the nocturnal peak (peak – nadir), mean 24-h leptin concentration, and 24-h AUC above the nadir on both HF/LC and LF/HC days. The correlation coefficients were generally higher with fat mass than with BMI or percent body fat. For example, on LF/HC and HF/LC days, fat mass was correlated with the leptin concentration at 0800 ($r = 0.67$, $P < 0.002$, and $r = 0.72$, $P < 0.0005$), the morning nadir ($r = 0.58$, $P < 0.01$, and $r = 0.62$, $P < 0.005$), the nighttime peak ($r = 0.78$, $P < 0.0001$, and $r = 0.71$, $P < 0.001$), the difference between the peak and nadir leptin concentrations ($r = 0.76$, $P < 0.0002$, and $r = 0.66$, $P < 0.002$), and the mean 24-h leptin concentration ($r = 0.74$, $P < 0.0005$, and $r = 0.66$, $P < 0.002$). The 24-h AUC was better correlated with fat mass on the LF/HC than on the HF/LC day ($r = 0.69$, $P < 0.001$, and $r = 0.47$, $P < 0.05$, respectively).

24-h plasma leptin profiles. Plasma leptin concentrations at 0800 and morning nadir leptin concentrations, which generally occurred at 1030–1100, were not different between the HF/LC and LF/HC study days (Table 4). There were significantly larger increases of leptin that occurred 4–6 h after breakfast and lunch on the LF/HC compared with the HF/LC day (Fig. 6*A* and *B*). The AUCs encompassing the postbreakfast (1000–1500) and postlunch (1500–2000) time periods were significantly smaller after HF/LC than LF/HC meals (Table 4). Peak nighttime leptin concentrations, the absolute magnitude of the nocturnal rise, and the percent change of leptin were all significantly smaller when subjects consumed HF/LC versus LF/HC meals (Table 4 and Fig. 6*A* and *B*). The nocturnal 12-h AUC was 22 ± 11% larger ($P < 0.05$) and overall leptin concentrations as assessed by the 24-h AUC were 38 ± 12% larger ($P < 0.0025$) on the LF/HC than the HF/LC day. Overall leptin secretion, as assessed by the 24-h AUC, was better correlated with peak nocturnal leptin concentrations than with either 0800 or morning nadir concentrations and was

TABLE 3
Cortisol concentrations before and after LF/HC or HF/LC meals in 19 women

Cortisol (nmol/l)	LF/HC	HF/LC
Baseline (0800)	700 ± 70	690 ± 70
Nighttime nadir	100 ± 20	100 ± 20
Morning peak	680 ± 60	700 ± 70
Peak – nadir	580 ± 50	600 ± 70
AUC 0800–0800 (mmol/l · 24 h)	5,355 ± 571	5,584 ± 585

Data are means ± SE. There were no significant differences between diets.

best correlated with the difference between the peak and nadir leptin concentrations (Table 5).

Correlations between leptin and cortisol responses. Although plasma leptin concentrations were low when plasma cortisol concentrations were high and vice versa, overall leptin responses (24-h AUC, mean 24-h concentrations, peak concentrations, amplitude of the peak minus the nadir, and percent change from peak to nadir) were not related to either the amplitude of the cortisol peak (r = 0.01–0.31, P = 0.99–0.20) or the 24-h AUC for cortisol on the LF/HC day (r = 0.04–0.15, P = 0.86–0.53). Parameters of leptin secretion and cortisol profiles were marginally correlated on the HF/LC day. The correlation coefficients and probability values for the relationships between the amplitude of the leptin peak and the 24-h AUC for leptin with the amplitude of the cortisol peak on the HF/LC day were r = 0.45, P = 0.0505, and r = 0.45, P = 0.053, and with the 24-h AUC for cortisol were r = 0.38, P = 0.1101, and r = 0.43, P = 0.0658.

DISCUSSION

This study was designed to examine the influence of dietary macronutrient content, specifically carbohydrate versus fat intake, on 24-h circulating leptin concentrations in women. We found that high-fat, low-carbohydrate meals, which produced smaller excursions of plasma insulin and glucose than low-fat, high-carbohydrate meals, resulted in reduced circulating leptin concentrations over a 24-h period. These data suggest that circulating leptin levels are not solely a reflection of adiposity or energy balance, but are also acutely affected by dietary macronutrient content. In previous studies, we and others found that fasting morning plasma leptin concentrations were not affected by dietary fat content in the absence of changes of body adiposity (9–11). One previous study found a relationship between changes of fasting plasma leptin and decreased carbohydrate, but not fat, intake during 28-day energy restriction in humans (29). The results of the present study indicate, however, that assessment of 24-h leptin profiles is necessary to reliably detect the effects of dietary macronutrient content on circulating leptin concentrations when energy intake is adequate.

Although fasting and morning nadir leptin concentrations are well correlated with adiposity, we found that these levels were not consistently related to 24-h leptin AUC (Table 5). Therefore, 24-h profiles are more informative than fasting levels for detecting changes of leptin secretion, as well as the amount of leptin to which the targets of leptin action, most notably the brain, would be exposed. Accordingly, studies that examine only fasting leptin concentrations under various physiological and pathophysiological conditions could overlook potential changes in the 24-h leptin profile. The most likely explanation why we and several other investigators (9–11) did not find an effect of varying the amounts of dietary fat and carbohydrate on circulating leptin is that in those studies circulating leptin concentrations were measured in the fasting state and in the morning, and not over a 24-h period with meal feeding. It is worth noting that amplitude of the nocturnal peak (the peak minus nadir) is highly correlated (r > 0.90) with the 24-h AUC (Table 5) and may serve as an index of 24-h leptin concentrations in studies in which 24-h blood sampling is precluded.

Meal feeding increases *ob* gene expression in rodents (30), and refeeding after fasting increases plasma leptin in humans (9,21); however, several studies have not found increases of leptin in response to meals in humans (9,31,32). For example, in a study by Weigle et al. (9), there was no increase of plasma leptin 5 h after a meal given at 0800 in men and women chronically consuming either a moderate-fat, moderate-carbohydrate diet or a low-fat, high-carbohydrate diet and no difference in leptin between the diets. In contrast, in our study conducted only in women, who have higher leptin levels than men (33), we did find a significantly larger change of leptin within 5 h after a LF/HC meal given at 0900 than after a HF/LC meal. Since the nadir of plasma leptin occurs between 1030 and 1100 (Fig. 6), an increase of plasma leptin 5 h after breakfast might not be observed if baseline concentrations are determined as 0800 values. If subjects do not eat, however, plasma

TABLE 4
Leptin concentrations before and after eating LF/HC or HF/LC meals in 19 women

Leptin (ng/ml)	LF/HC	HF/LC	P
Baseline (0800)	12.7 ± 1.9	12.8 ± 1.8	NS
Morning nadir	10.7 ± 1.6	10.9 ± 1.8	NS
Nighttime peak	21.3 ± 2.5	19.9 ± 2.6	<0.05
Peak – nadir	10.6 ± 1.3	9.0 ± 1.1	<0.01
Percent change (nadir to peak)	122.5 ± 16.2	95.8 ± 7.3	<0.025
AUC 1000–1500 (ng/ml · 5 h)	11.7 ± 2.0	4.7 ± 1.0	<0.0005
AUC 1500–2000 (ng/ml · 5 h)	29.8 ± 4.3	17.2 ± 3.1	<0.0025
AUC 2000–0800 (ng/ml · 12 h)	84.5 ± 11.7	72.2 ± 9.7	<0.05
AUC 0800–0800 (ng/ml · 24 h)	128.9 ± 17.2	97.4 ± 12.8	<0.01

Data are means ± SE.

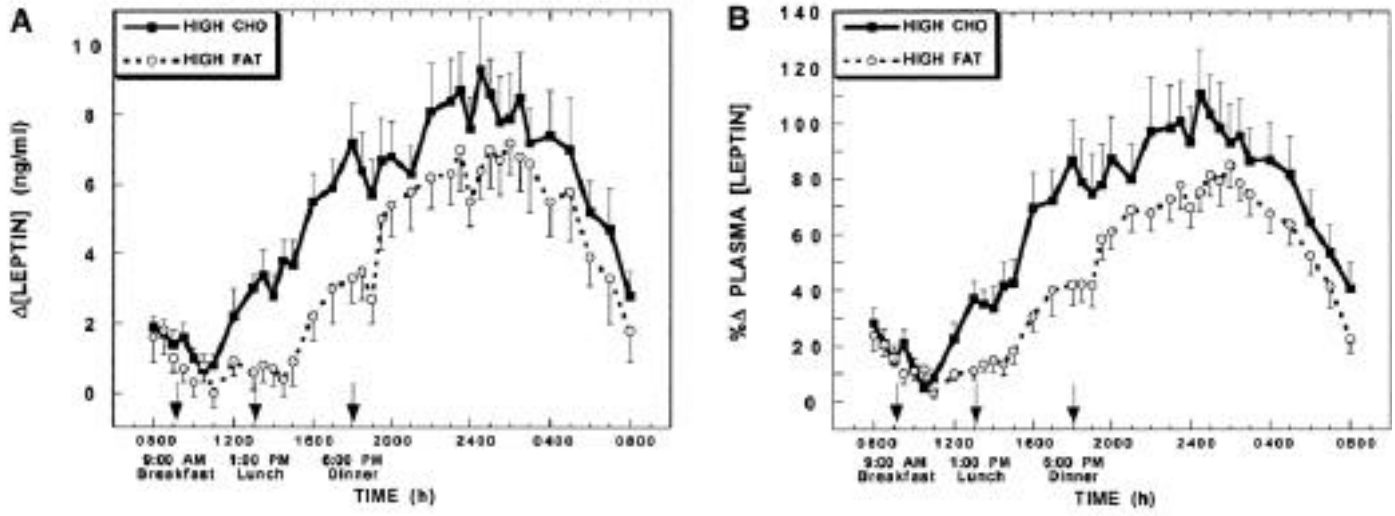

FIG. 6. *A*: **The absolute change of plasma leptin concentrations over morning nadir concentrations during a 24-h period (0800–0800) in 19 women consuming three LF/HC or three HF/LC meals.** *B*: **The percent change of plasma leptin concentrations over morning nadir concentrations during a 24-h period (0800–0800) in 19 women consuming three LF/HC or three HF/LC meals.**

leptin concentrations do not increase and in fact continue to fall from the nadir (18). Thus, a lack of a significant increase after a meal should not be interpreted to indicate that the meal had no effect on circulating leptin.

We observed larger increases of leptin 4–6 h after high-carbohydrate meals than after high-fat meals. These responses are consistent with previous observations that leptin increases within 4–6 h after exogenous insulin or glucose administration in humans and nonhuman primates (34–40). The increased leptin concentrations with high-carbohydrate feeding may result from an effect of insulin and glucose excursions after high-carbohydrate meals to increase glucose uptake and metabolism in adipose tissue, since glucose utilization has recently been shown to have a major influence on leptin gene expression and on leptin secretion from adipocytes in vitro (23). It is possible that the progressive increase of leptin over the course of a day reflects a cumulative effect of insulin secreted and glucose absorbed and metabolized after each meal. It is therefore of interest that pulsatile leptin secretion has been reported to increase several hours after meals and is related to meal-induced insulin secretion (14).

We did not find a strong relationship between circulating cortisol and leptin responses. Although several studies have also found an inverse temporal relationship between circulating cortisol and nocturnal leptin excursions (13,26,27), it is unlikely glucocorticoids have a major role in regulating the nocturnal rise of leptin secretion, particularly in light of reports that delaying meal feeding for 6.5 h acutely shifts the timing of the nocturnal leptin peak by an equivalent time period without changing the circadian cortisol rhythm. We believe it is more likely that cortisol modulates leptin secretion indirectly via its effects to decrease insulin sensitivity. For example, cortisol may be involved in the diurnal rhythm of circulating leptin concentrations that has been observed during long-term hyperinsulinemic-euglycemic clamps in fasting subjects (41). In that study, the changes of leptin concentrations have a similar 24-h periodicity as changes of insulin sensitivity, as assessed by the changes in the glucose infusion rate.

There is increasing evidence that leptin is an important determinant of energy balance in humans (24). Genetic impairment of leptin production (25,42) or defects in the leptin receptor (43) have been shown to result in hyperphagia and massive obesity in humans. Low leptin levels were predictive of later weight gain in Pima Indians (44), and nocturnal increases of serum leptin were inversely related to weight gain in adolescent females (45). In addition, we have found that decreases of leptin are related to increased hunger sensations during moderate prolonged energy restriction in women (46), and that central administration of leptin inhibits food intake and activates the sympathetic nervous system in rhesus monkeys (47).

Decreased leptin secretion during consumption of diets high in fat would decrease the overall amount of leptin to which the central nervous system is exposed and could therefore contribute to the adipogenic effects of high-fat diets in animals and humans (1–6). This effect could be mediated by leptin's actions to decrease appetite and/or increase energy expenditure. Conversely, increased leptin production induced by dietary carbohydrate may contribute to the effects of low-fat diets to induce weight loss even when such diets are consumed ad libitum (11,48). In a previous study, we found that energy intake needed to be increased by 6% (120 ± 30 kcal/day) to maintain body weight in women when dietary fat content was lowered from 31 to 14% and replaced with carbohydrate (11). Furthermore, when fat or carbohydrate is fed in excess of energy requirements for 14 days, a greater

TABLE 5
Correlations of 24-h leptin AUCs with plasma leptin concentrations in 19 women consuming LF/HC or HF/LC meals

	LF/HC		HF/LC	
Leptin (ng/ml)	*r*	*P*	*r*	*P*
Baseline (0800)	0.39	0.0947	0.56	0.0128
Morning nadir	0.30	0.2059	0.40	0.0880
Nighttime peak	0.67	0.0016	0.66	0.0021
Peak – nadir	0.90	0.0001	0.92	0.0001

amount of the surplus energy from fat (90–95%) is stored than from carbohydrate (75–85%) (1). These results suggest that increasing dietary carbohydrate content alters the regulated level of adiposity by increasing energy expenditure or altering nutrient partitioning. These effects could be mediated by increased leptin production as well as by the higher energetic costs of storing ingested carbohydrate as fat. The possibility that changes of circulating insulin concentrations may directly contribute to the effects of dietary macronutrient content on adiposity should also be considered, since insulin is likely to be involved in regulating energy balance via a direct action within the central nervous system (49,50).

In summary, consumption of high-fat, low-carbohydrate meals results in lower circulating leptin concentrations over a 24-h period in women than consumption of low-fat, high-carbohydrate meals. The reduction of leptin secretion after high-fat meals may be a consequence of decreased insulin release after meals, leading to lowered glucose metabolism in adipose tissue. Although the fat content of the high-fat meals in this study was quite high, many meals from fast-food restaurants contain 60% or more of energy from fat. Therefore, reduced overall leptin responses would be expected in people consuming such high-fat meals in a free-living situation. We hypothesize that decreases of circulating leptin concentrations when high-fat diets are consumed may contribute to the effect of high-fat foods to induce weight gain, whereas increases of circulating leptin could contribute to the weight-reducing effect of high-carbohydrate, low-fat diets.

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