High Glycemic Index Carbohydrate Diet Alters the Diurnal Rhythm of Leptin But Not Insulin Concentrations

TRACY S. HERRMANN, MICHELLE L. BEAN, TRACY M. BLACK, PING WANG, AND ROSALIND A. COLEMAN[1]

Department of Nutrition, University of North Carolina, Chapel Hill, North Carolina 27599

Morning serum leptin values in humans are inconsistently altered by diet, and the molecular mechanisms controlling the diurnal leptin pattern remain unexplained. We determined whether leptin values after meals or the leptin diurnal pattern was altered by the type of carbohydrate (CHO) ingested in diets containing either 20% or 30% fat. In a randomized, crossover study design, nine healthy lean adults ate one of four isocaloric diets for 8 days. Diets contained 15% protein: A, high glycemic index (GI) CHO, 30% fat; B, low GI CHO, 30% fat; C, high GI CHO, 20% fat; and D, low GI CHO, 20% fat. Serum glucose, insulin, and leptin were measured at intervals on Day 8 for 24 hr, and on Day 9 during an oral glucose tolerance test (GTT). Although the 24-hr glucose and insulin profiles did not differ with the diets, diets A and C altered the serum leptin diurnal pattern. In contrast to the usual evening rise in leptin concentration, which begins after 2200 hr, diets A and C caused a rise in leptin beginning at 1300 hr. The area under the curve for leptin between 1230 and 2400 hr was 17% greater for diets A and C. During the GTT, leptin concentrations were similar for each diet. These results suggest that the pattern and amount of leptin secretion may be altered by high GI CHO or the simple sugar content of the diet, unrelated to differences in insulin concentration, that high GI foods may have little or no effect on serum insulin in the context of a mixed meal, and that a single 0800-hr leptin value may not be sufficient to reveal a diet-induced change in leptin secretion [Exp Biol Med Vol. 226(11):1037–1044, 2001]

Key words: leptin; insulin; glucose; high glycemic index carbohydrate; satiety; sucrose; diurnal rhythm

Leptin, a 16-kDa protein encoded by the *ob* gene is believed to convey information about energy stores to the brain and thereby regulate satiety, food intake, and energy expenditure (1, 2). Administration of leptin to leptin-deficient *ob/ob* mice decreases food intake, body weight, and adiposity while increasing metabolic rate, body temperature, and level of activity (3–6), and leptin administration to a child congenitally deficient in leptin decreased food intake and weight and increased activity (7). Leptin also alters energy regulation in muscle and pancreatic islet cells, attenuating insulin's lipogenic action by increasing fatty acid oxidation, and decreasing triacylglycerol synthesis (8, 9). These profound effects on appetite, metabolism, and body weight have provoked an interest in how leptin is regulated.

Basal fasting leptin values are strongly and positively related to serum insulin, gender, pregnancy, percent body fat, and body mass index (BMI) (10, 11). Although plasma leptin concentrations correlate with adipose tissue mass in animals (12) and humans (13–16), the marked changes in leptin that occur with short-term fasting or feeding do not correspond to the amount of fat mass lost or gained (17–21). This discrepancy suggests that factors other than adipocyte size and content must influence leptin production. Because a large fat mass still exists after a 3-day fast, marked decreases in serum leptin suggest that the leptin concentration might reflect intracellular nutrient or energy availability rather than the actual amount of triacylglycerol stores in the adipocyte. Studies using energy-producing substrates and various channel blockers indicate that potassium and calcium may couple energy production to the secretion of leptin (22), and leptin expression has been induced in mouse skeletal muscle by increases in serum glucose or fatty acid and in 3T3-L1 preadipocytes and L6 myocytes by incubations with glucosamine, suggesting a link to increased energy availability (23). How cells might sense changes in nutrient availability, however, is unknown. Further, the regulation of leptin's diurnal pattern remains unexplained (16, 24, 25).

Insulin appears to mediate between the nutritional state

This study was supported by a grant from Mars, Inc. and by General Clinical Research Center (grant M01 RR00046) and Clinical Nutrition Research Center (grant DK56350) from the National Institutes of Health

[1] To whom requests for reprints should be addressed at Departments of Nutrition and Pediatrics, CB #7400, University of North Carolina, Chapel Hill, NC 27599. E-mail: rcoleman@unc.edu

Received March 14, 2001.
Accepted July 31, 2001.

1535-3702/01/22611-1037$15.00

and leptin secretion in both rodents and humans, but effects in rodents occur within a shorter time frame. Postprandial hyperinsulinemia restores leptin mRNA abundance in rat adipose tissue within 4 hr after a fast, and a single insulin injection in fasted rats increases adipose leptin mRNA abundance to levels similar to those of fed controls (26, 27). Adipose leptin mRNA in normal rats increases in parallel with rising hyperinsulinemia (26), and insulin increases leptin mRNA expression and leptin secretion from rat adipocytes (28). In humans, however, insulin affects serum leptin less acutely. A euglycemic hyperinsulinemic clamp prevents the usual mid-morning drop in serum leptin, but requires 4–6 hr before leptin levels increase over basal values (29–33). Human adipocyte leptin mRNA abundance did not change after a 3-hr euglycemic hyperinsulinemic clamp (34), and mRNA increases inconsistently after insulin exposure (35–37).

Although insulin may mediate the positive correlation between body fat and leptin, human studies have yielded varying results. In normal weight and obese women who continued weight-maintenance diets that contained 14%, 23%, or 31% of energy as fat, leptin concentrations did not change from baseline or vary between diets (14). Leptin concentrations did not change in adults who ate a high fat diet (60% of energy as fat) (13). In each of these studies, however, leptin was measured at a single morning time point; thus, neither peak values nor the diurnal pattern was ascertained. In contrast, leptin's diurnal pattern differed markedly in each arm of a crossover study with three isocaloric meals that contained markedly varying amounts of energy as carbohydrate and fat (38).

The present study was designed to examine the effects of diets that contained either high- or low-glycemic index (GI) carbohydrates in the context of either 20% or 30% energy from fat on 24-hr serum profiles of leptin, insulin, and glucose and on hormonal responses to a glucose tolerance test (GTT).

Materials and Methods

Subjects. Healthy adults with BMIs lower than 26 kg/m^2 were recruited from the Chapel Hill community. The final study sample included five males and four females, including one black, six white, and two Asian subjects. Data from an additional subject was not used in the analysis because she did not comply with the study protocol. For all subjects, the physical examination and laboratory screens (CBC, urinalysis, urine drug screen, and HbA1C) were normal. All subjects were interviewed and examined by a physician. They were free of disease, including diabetes and hypertension, and were not trying to lose weight, taking drugs to treat obesity, or eating special or restricted (nutrient or energy) diets. No subjects were taking oral contraceptives, thiazides, beta-blockers, psychotropic drugs, or any drugs known to alter carbohydrate metabolism. No female subjects were postmenopausal or pregnant. All denied using illicit drugs and refrained from alcohol during the study periods. Subjects were lean (mean BMI was 22 kg/m^2 for women and 23.6 kg/m^2 for men), nonsmoking, and between the ages of 19 and 36 y (Table I).

Experimental Protocol. The protocol was approved by the Institutional Review Board of the University of North Carolina. Each subject gave informed, written consent to participate. All investigative procedures were conducted in the General Clinical Research Center (GCRC) at UNC Hospitals. Subjects were tested in a randomized, crossover study design. Subjects continued their usual activities and exercise patterns during the study. Metabolic testing began after a 7-day intake of four isocaloric diets that differed in fat and carbohydrate content. Subjects were randomly assigned to the following diets: A, high GI carbohydrate (CHO), 30% fat; B, low GI CHO, 30% fat; C, high GI CHO, 20% fat; and D, low GI CHO, 20% fat. Each diet contained 15% protein. During each diet period, subjects were served breakfast and dinner in the GCRC as outpatients, and were given a lunch and snack to eat at home. Subjects checked off foods eaten in the lunches and snacks, and GCRU staff monitored compliance on the GCRC. By observation or report, all food was consumed. On the eighth day of each study diet, the subjects were admitted to the GCRC and stayed in rooms without televisions (to avoid eating-related cues). Meals were brought to them at 0800, 1200, and 1800 hr, and snacks were offered at 1600 hr (test snack) and 2200 hr. Activity was limited. An indwelling i.v. catheter was placed at 0700 hr to draw blood.

Diets. Each subject consumed the four diets (A, B, C, and D) for 8 d in a randomized, crossover study design. Isocaloric diets were determined for each subject based on height, weight, activity level (Harris and Benedict equations), a 24 hr recall, and a 3-day food record. To overcome possible underreporting, diets were calculated with a 10% increase over reported records based on the experience of subjects who were still hungry (39). Subjects checked off foods that were eaten. Weights remained constant throughout the study. Diets were created using the Diet Planner

Table I. Baseline Anthropometric and Biochemical Characteristics of All Subjects[a]

	Men (n = 5)	Women (n = 4)
Age (years)	29.8 ± 6.5	23.3 ± 3.6
(Range)	(22–37)	(20–28)
BMI (kg/m^2)	23.6 ± 2.9	22.0 ± 3.4
(Range)	(20–26)	(18–26)
Body fat % (TSF)[b]	11.6 ± 2.9	20.3 ± 5.6
(Range)	(7.7–15.3)	(15.7–27.5)
Hgb A1C (%)	5.5 ± 0.3	5.0 ± 0.3
Diet A [0800 leptin (ng/ml)[c]]	3.63 ± 2.2	8.91 ± 4.8
Diet B [0800 leptin (ng/ml)[c]]	4.87 ± 3.2	7.28 ± 3.1
Diet C [0800 leptin (ng/ml)[c]]	3.33 ± 1.8	9.47 ± 4.6
Diet D [0800 leptin (ng/ml)[c]]	3.65 ± 2.1	8.67 ± 4.8

[a] Values are reported as the mean ± SD.
[b] TSF (triceps skinfold measurement in triplicate).
[c] 0800 values on Days 8 and 9 were averaged for each subject eating each diet.

software, developed for Clinical Research Centers at the University of California (version 2.1, San Francisco, CA). Nutrient calculations were performed using the Minnesota Nutrition Data System (NDS) software, developed by the Nutrition Coordinating Center at the University of Minnesota (version 2.91, Minneapolis, MN). Meal plans were individualized for personal taste, yet conformed to the macronutrient distribution of the study design. (For example, an increased amount of chicken and rice was provided for a subject who did not drink milk, and substitutes were made for disliked vegetables.) Total energy for each day was distributed as 20% for breakfast, 25% for lunch, 35% for dinner, and 10% each for an afternoon and an evening snack. The International Table of Glycemic Index Foods was used to determine GIs of the foods (40). The published GI values for food are based on studies of blood glucose levels following the intake of equal carbohydrate portions of different foods. GI values of foods were determined by comparing blood glucose levels after the intake of each food to blood glucose levels after 50 g of glucose. The standard 50 g of glucose yields a score of 100, whereas sugars like sucrose and fructose produce mean scores of 60 and 23, respectively. The low GI CHO diets (B and D) contained foods with a GI score of ≤ 45 and the high GI CHO diets (A and C) contained foods with a GI score of >65. Inpatient diets on Day 8 followed the same menus with micro- and macro nutrient compositions similar to the outpatient diets. Subjects underwent a minimum washout period of 10 days between study diets.

Satiety Measurements. To assess satiety, subjective criteria such as the desire to eat, feeling of hunger, feeling of fullness, and prospective feeding intentions were measured using a 100-mm visual analogue scale (VAS) (41). The VAS was designed to obtain subjective satiety levels by asking the subjects to score their answers to four questions on a scale from 1 to 10 based on the level their perceived satiety. The questions were: "How strong is your desire is to eat? How hungry do you feel? How full do you feel? and How much food do you think you could eat?". Due to the consistent responses to all four questions and the similar information gathered from each question, the mean scores for all four questions were combined (with the "How full do you feel?" question plotted in the reverse direction) to analyze the data for each of the four diets. Lunch was eaten between 1200 and 1230 hr. The questionnaire was administered at 1230 hr and every 30 min until 1600 hr when a standard test snack of canned peaches (300 g) in natural juice (GI = 30) was offered. Although we had hoped to measure satiety objectively by quantifying the amount of the test snack eaten, the snack offered was too small and all subjects ate 100% of all test snacks.

Leptin, Insulin, and Glucose Profiles. Blood was collected for glucose, insulin, and leptin concentrations beginning at 0800 hr on the eighth study day and at intervals throughout the next 30 hr. Intervals were every 30 min for 2 hr following meals, every 60 min at other times during the day, and every 120 min between 2200 and 0800 hr. On the ninth day at 0800 hr, following a 12-hr fast, an oral GTT was performed using 75 g of glucose (Limondex). Serum glucose, insulin, and leptin were measured at 0755 hr (baseline), and at 0830, 0900, 0930, 1000, 1100, and 1200 hr during the GTT. Blood samples were centrifuged within 30 min of collection and the serum was aliquoted and stored at −20°C for insulin and leptin assays. Glucose was measured immediately by the University of North Carolina Hospitals clinical laboratory. Serum insulin and leptin concentrations were measured in duplicate using commercially available radio immunoassays from Diagnostic Systems Labs (Webster, TX [insulin]) and Linco Research (St. Charles, MO [leptin]). Technicians were blinded to the experimental conditions.

Statistical Analysis. Areas under the curve (AUC) were calculated for glucose, insulin, and leptin with a trapezoidal method using an algorithm that multiplies the mean serum concentration at a pair of adjacent time points by the corresponding time interval and takes the sum of the AUC. For each subject, the AUC for leptin, insulin, and glucose are calculated. The AUC values for glucose and insulin are calculated using milligrams per decaliter or micro-units per milliliter, respectively; for leptin, we used the percentage of the baseline levels taken at 0755 hr. AUC values are expressed as units per hour for the first 24 hr of sampling, for the interval between 1230 and 2400 hr, and during the 4-hr GTT.

To determine the effect of diet on leptin, insulin, and glucose over time, mathematical models were developed using the General Linear Model procedure for regression analysis in SAS (SAS 6.12, Cary, NC). Mathematical models were used to fit changes in serum concentrations over time in order to allow for multiple bends in the curve. The shapes of the curves were fit by adding time exponentially to the linear models until a model was found in which the last time to the highest power was not significant. The model in which the highest power of time was significant was used to test the experimental manipulations. Individual models, including a term for subject effect, were created to analyze glucose, insulin, and leptin. For example, the first model for leptin analyzed values between 1230 and 2400 hr using time to the fifth power, the second model for leptin analyzed values during the first 24 hr using time to the fourth power, and the third model for leptin analyzed values during the 4-hr GTT using time to the third power. Tukey's *post hoc* test was used to determine which diets differed from each other in their effect on the serum concentrations of leptin, insulin, and glucose over time. Satiety levels were calculated by taking the mean score of four questions on the VAS questionnaire. Data were also analyzed using the General Linear Model procedure and Tukey's *post hoc* test in SAS. No significant differences were found except for the linear value of time. Results are presented as means ± SE. Significance was defined as $P < 0.05$.

Results

Glucose, Insulin, and Leptin Profiles. Despite the differences in the percentage of CHO in each diet or the presence of high or low GI CHO, serum glucose and insulin profiles did not differ for each of the diets and there were no statistically significant differences for peaks or troughs (Fig. 1). The AUCs for insulin and glucose were not significantly different for the four diets (Table II). The basal leptin values were not significantly different for any of the diets (Table I). With the two low GI CHO diets, B and D, the 24-hr leptin profile (Fig. 2) was similar to that of other reports (25). As was previously shown in both lean and obese women (25), leptin concentrations remained low throughout the day, rose above the baseline (0800 hr) values after 2300 hr, peaked at 0200 hr, and returned to baseline by 0800 hr. In contrast, the high GI CHO diets, A and C, altered the 24-hr leptin profiles. Serum leptin rose above the 0800 hr baseline as early as 1300 hr, whereas leptin concentrations with diets B and D did not rise above baseline until 2400 hr. The leptin AUCs from 1230 hr to 2400 hr for diets A and C were significantly higher than for diets B and D ($P < 0.0001$; Table II) using the first model. Leptin AUCs for all diets peaked at 0200 hr, and there were no significant differences between the peak heights attained. Leptin AUCs for diets A and C began to decrease after 0200 to 0400 hr, yet did not fall as rapidly as did leptin AUCs on diets B and D. The 24-hr AUCs for leptin on diets A and C were significantly greater than the AUCs for leptin on diet B ($P < 0.003$) using the second model. We examined the effect of gender on leptin in response to the four different diets and found no statistically significant differences between males and females.

GTT. During the GTT, serum glucose and insulin concentrations rose similarly for subjects who had eaten each of the four diets during the preceding 8 days (Fig. 1), and the GTT AUC for insulin and glucose were not significantly different between diets (Table II). Diet C (high GI CHO, 20% fat) caused the leptin AUC to rise significantly higher than diet D (low GI CHO, 20% fat) during the GTT ($P < 0.01$) using the third model; however, the starting leptin value was also elevated.

Satiety Test. Satiety levels did not differ significantly after the four diet lunches (data not shown). Each subject felt minimally hungry immediately after lunch (score 2.5–3.3), and hunger increased moderately during the next 3.5 hr (final score 5.5–6.3).

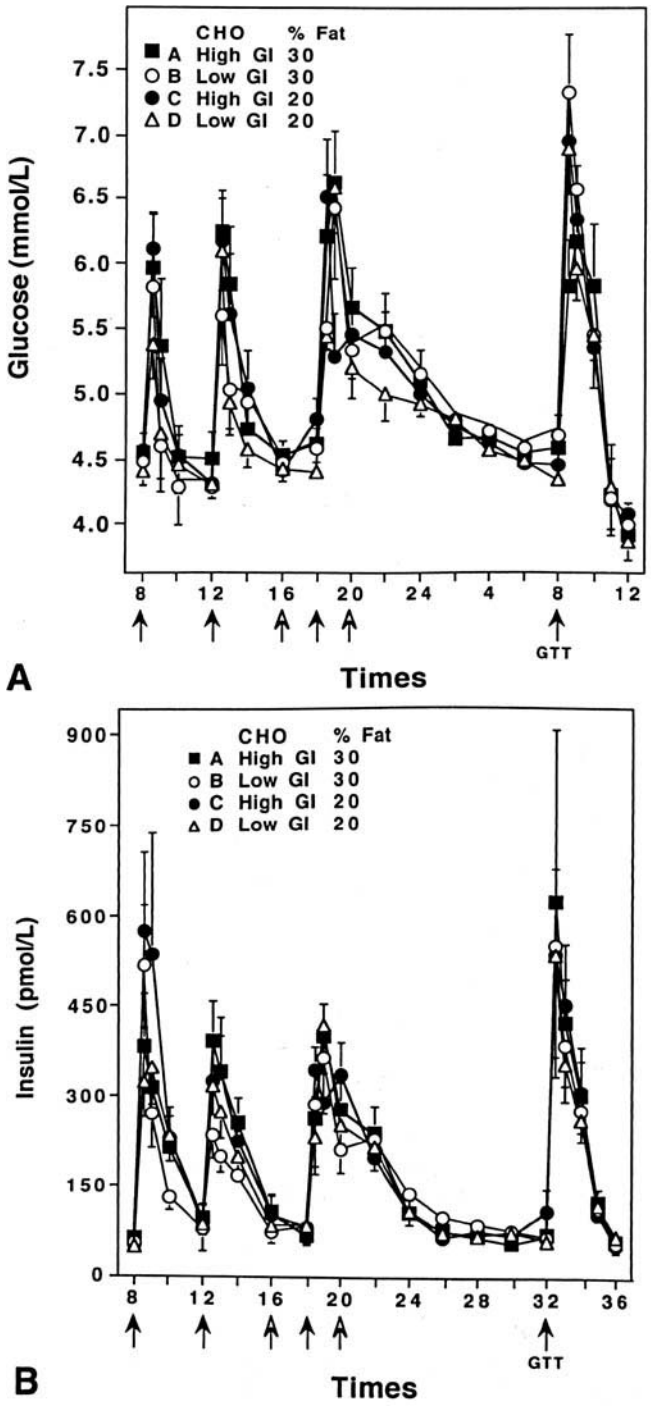

Figure 1. Serum glucose (A) and insulin (B) concentrations for healthy adults eating each of four experimental diets during a 28-hr period. Each point represents the mean ± SE for eight to nine samples. Solid arrows indicate meals and ingestion of glucose for the GTT. Open arrows indicate snacks.

Discussion

Although initially identified as a satiety hormone because of the gross obesity observed in genetic human and rodent leptin deficiencies, it has been hypothesized that leptin functions to alter energy metabolism during starvation (1) and that it has additional functions in reproduction and in the immune system (42). Thus, in nonobese people, an increase in serum leptin may signal that energy stores are replete, whereas a decrease in leptin may signal the need for food intake (43). Although leptin secretion is positively related to adipose tissue mass, serum leptin decreases markedly with minor fasting that has little effect on total adipose mass, (20) suggesting that leptin secretion depends on factors that signal recent energy intake (18, 20, 21). Insulin is one of these factors, but independent of insulin's effect on

Table II. Glucose, Insulin, and Leptin Areas Under the Curve (AUC) for Four Experimental Diets Given to Healthy Adults

	Diet A	Diet B	Diet C	Diet D	P value[b]
Glucose, Day 8					
0800–0800 hr	16.9 ± 0.6	16.5 ± 0.6	16.6 ± 0.6	16.1 ± 0.6	NS
AUC mmol/L/hr (mg/dl/hr)	[304 ± 11]	[297 ± 11]	[298 ± 11]	[290 ± 11]	
Glucose, glucose tolerance test (GTT)					
0800–1200 hr	63.3 ± 3.4	66 ± 2.4	64.4 ± 2.4	63.7 ± 2.6	NS
AUC mmol/L/hr (mg/dl/hr)	[1141 ± 61]	[1189 ± 43]	[1161 ± 44]	[1147 ± 47]	
Insulin, Day 8					
0800–0800 hr (AUC μIU/ml/hr)	79 ± 4	70 ± 4	83 ± 5	74 ± 4	NS
Insulin, GTT					
0800–1200 hr (AUC μIU/ml/hr)	474 ± 61	429 ± 49	459 ± 55	414 ± 43	NS
Leptin, Day 8					
0800–0800 hr (% baseline–AUC/hr)	368 ± 17[c]	328 ± 15[d]	377 ± 18[c]	340 ± 16[c,d]	$P < 0.003$
Leptin					
1230–2400 hr (% baseline–AUC/hr)	731 ± 47[c]	624 ± 49[d]	751 ± 47[c]	643 ± 41[d]	$P < 0.0001$
Leptin, GTT					
0800–1200 hr (% baseline–AUC/hr)	1192 ± 66[c,d]	1157 ± 55[c,d]	1243 ± 61[c]	1082 ± 47[d]	$P < 0.01$

Note. Diet A, high GI CHO, 30% fat; Diet B, low GI CHO, 30% fat; Diet C, high GI CHO, 20% fat; and Diet D, low GI CHO, 20% fat.[a]
[a] Values are reported as the mean ± SE.
[b] General Linear Models procedure in SAS.
[c,d] Tukey's post hoc test for differences between diets with $\alpha = 0.05$.

leptin, energy-producing substrates and fluxes of calcium and potassium are also required (22).

If a rise in leptin suppresses appetite, the higher serum leptin levels between 1200 and 1600 hr for diets A and C should have increased satiety compared with diets B and D. However, the results from the VAS satiety scale demonstrated that satiety did not differ between diets. These data are consistent with another study that showed that leptin did not have an acute effect on satiety (44).

Neither the satiety nor the starvation prevention hypotheses address the question of why leptin values peak nocturnally or what molecular mechanism regulates leptin's diurnal rhythm. Leptin's 24-hr pattern shows a nadir between 0900 and 1400 hr, and a peak between 2400 and 0200 hr (25, 45). However, most studies that have examined diet influences on leptin levels have relied on a single fasting measurement obtained around 0800 hr when serum leptin concentrations are declining from their nocturnal peak, but are still 20% higher than their lowest daily value.

Basal leptin values in our subjects were similar for all

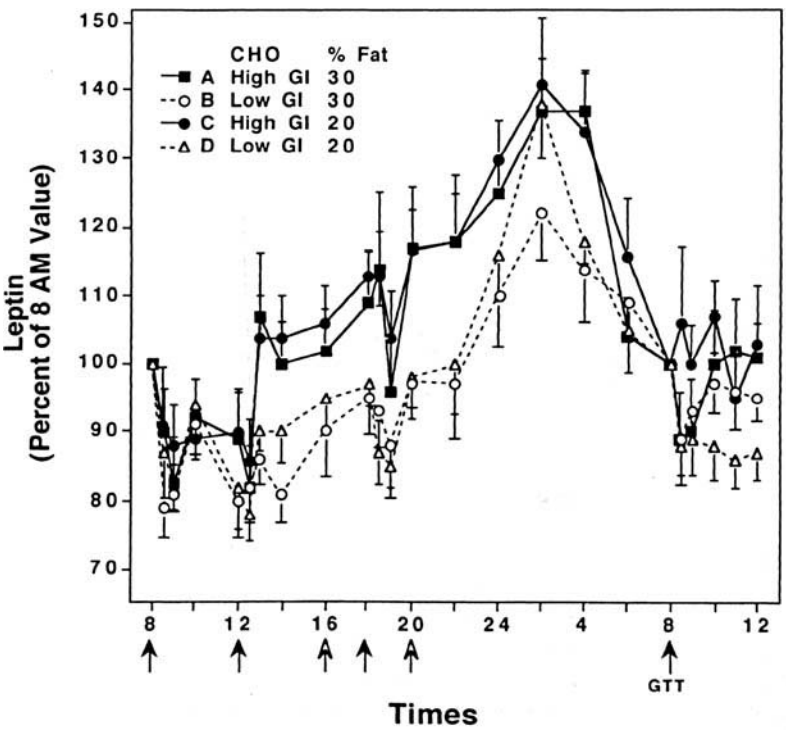

Figure 2. Percent change in serum leptin concentrations for healthy adults eating each of four experimental diets during a 28-hr period. Leptin values are plotted as percentages because of the marked differences between values from men and women. The two 0800-hr values were averaged and taken as 100%. Each point represents the mean ± SE for eight to nine samples. Solid arrows indicate meals and ingestion of glucose for the GTT. Open arrows indicate snacks.

Table III. CHO Content of Meals for Each of Four Diets from the Evening before the Study Day and the Breakfast and Lunch of the Study Day[a]

A. Dinner/evening snack, Day 7: CHO intake (g)

Diet	Fat (%)	Glycemic index	Starch	Sucrose	Glucose	Fructose	Lactose	Total glucose	Glucose from sugars	Total fructose	Total CHO
A	30	High	31	51	5	6	8	65.5	34.5	31.5	101
B	30	Low	89	3	6	12	0.4	96.7	7.5	13.5	110.4
C	20	High	30	54	10	10	12	73	43	37	116
D	20	Low	122	2	3	3	0.3	126	4	4	130.3

B. Breakfast, Day 8: CHO intake (g)

Diet	Fat (%)	Glycemic index	Starch	Sucrose	Glucose	Fructose	Lactose	Total glucose	Glucose from sugars	Total fructose	Total CHO
A	30	High	26	3	5	4	11	38	12	5.5	49
B	30	Low	12	2	14	12	11	32.5	20.5	13.0	51
C	20	High	24	16	4	2	10	41	17	10.0	56
D	20	Low	22	1	12	11	11	40	18	11.5	57

C. Lunch, Day 8: CHO intake (g)

Diet	Fat (%)	Glycemic index	Starch	Sucrose	Glucose	Fructose	Lactose	Total glucose	Glucose from sugars	Total fructose	Total CHO
A	30	High	43	20	7	3	0.1	60	17	13	73
B	30	Low	11	12	11	22	0.3	28	17	28	56
C	20	High	11	49	14	6	0.1	49.5	39	30.5	80
D	20	Low	11	5	18	31	0.3	31.5	21	33.5	65

D. Total CHO intake for 24 hr before the 1300 hr rise in leptin concentration: Day 7 dinner/evening snack plus Day 8 breakfast and lunch (g)

Diet	Fat (%)	Glycemic index	Starch	Sucrose	Glucose	Fructose	Lactose	Total glucose	Glucose from sugars	Total fructose	Total CHO
A	30	High	100	74	17	13	19	163.5	63.5	50	223
B	30	Low	112	17	31	46	11	157	45	54.5	217
C	20	High	65	119	28	18	22	163.5	98.5	77.5	252
D	20	Low	155	8	33	45	12	198	43	49	253

E. Total CHO intake: Day 8 (g)

Diet	Fat (%)	Glycemic index	Starch	Sucrose	Glucose	Fructose	Lactose	Total glucose	Glucose from sugars	Total fructose	Total CHO
A	30	High	132	60	15	13	21	187.5	57	43	241
B	30	Low	82	31	38	53	12	141.5	59	68.5	216
C	20	High	108	122	27	22	11	201.5	93.5	83	290
D	20	Low	105	26	44	71	12	168	63	84	258

Note. Diet A, high glycemic index (GI) carbohydrate (CHO), 30% fat; Diet B, low GI CHO, 30% fat; Diet C, high GI CHO, 20% fat; Diet D, low GI CHO, 20% fat.

[a] CHO amounts are given in grams (Minnesota Data system 2.91). Total fructose, free fructose plus one-half sucrose in grams. Total glucose, starch + one-half sucrose and one-half lactose in grams. Glucose from sugars, glucose + one-half sucrose + one-half lactose in grams. For Total CHO, apparent differences in summing the individual CHO components arise because not all diet carbohydrates are included (e.g., maltose). Note that the Total CHO reflects individual meals, not daily totals.

four diets, showing that reliance on this one measure would have suggested that the diets had no effect. By measuring leptin over a 24-hr period, however, our data revealed that both diets that contained high-GI CHOs altered the 24-hr leptin profile and increased the 24-hr leptin AUC. Instead of serum leptin concentrations remaining low throughout most of the day, with both the high GI diets, leptin increased above the 0800 hr baseline by 1300 hr and, aside from a dip at 1900 hr, remained elevated until 0400 hr the next morning. This early and prolonged rise resulted in leptin AUCs that were 12% higher for the 24-hr period and 17% higher between 1230 and 2400 hr for diets A and C compared with diets B and D. However, basal and meal-related serum glucose and insulin concentrations were similar when the percentage of energy from fat was 20% or 30% and the corresponding CHO was 65% or 55%, or when the diets contained high or low GI CHOs.

Although fasting for as little as 24 hr eliminates leptin's

nocturnal rise (18), only two studies have demonstrated that meal timing or diet composition can alter leptin's diurnal rhythm. Delaying meals for 6.5 hr shifts the plasma leptin pattern by 5–7 hr within 3 days (45). The three meals given were isocaloric, and the authors speculated that providing more calories early in the day caused the nocturnal rise in leptin to occur earlier than had been previously reported (25). The second study that altered leptin's 24-hr pattern provided three isocaloric meals that contained either 60% of energy as CHO and 20% of energy as fat or 20% of energy as CHO and 60% of energy as fat (38). As would be expected with diets that vary greatly in their CHO content, serum glucose and insulin values were higher after each of the high CHO meals and the AUC for both glucose and insulin were higher with the 60% CHO diet. For leptin, the 24-hr AUC for the 24-hr leptin profile was 38% larger and was attributed to the marked increase that had occurred in serum insulin.

In our study, neither the amount of CHO in the diet nor the serum insulin response are responsible for the altered 24-hr leptin profile because the same amount of CHO was present in diets A and B (55%) and in diets C and D (65%), and insulin profiles after meals and insulin AUC did not differ for the four diets. In the context of mixed meals, some studies have shown differences in insulin following high and low GI CHO, whereas other studies have not (46). Because our study provided only 20% of the day's calories at 0800 hr, early provision of increased calories did not play a role. Thus, it seems that factors other than insulin might regulate the 24-hr leptin profile.

If high GI CHO alter the 24-hr serum leptin pattern and amount without affecting serum glucose or insulin values, what is the mediator? The hexosamine biosynthetic pathway is believed to sense energy availability and mediate the effects of glucose on the expression of gene products such as growth factor-α in vascular smooth muscle cells (47, 48). A direct signaling link between UDP-*N*-acetylglucosamine (UDP-GlcNAc) and leptin mRNA expression was demonstrated in 3T3-L1 preadipocytes, adipose tissue, skeletal muscle, and L6 myocytes (23). In this study, glucosamine, glucose, and fatty acids, which increase the hexosamine pathway, promoted leptin expression.

Analysis of the carbohydrates used in our study showed two differences between diets A and C and diets B and D: the sucrose content and the content of glucose provided from sugars (glucose, sucrose, and lactose; Table III). For example, on Day 8 for a representative 55-kg woman, diets A and C contained 60 and 122 g of sucrose, respectively, whereas diets B and D contained 31 and 26 g, respectively (Table IIIE). Similar marked differences in sucrose content were present in all diets eaten by our study participants. Thus, during the 19 hr preceding the leptin rise, diets A and C provided 4.4–14.8 times more sucrose than did diets B and D. A study of diets that differed markedly in starch and sucrose showed large differences in serum glucose, insulin, and related metabolites (49), but we did not observe similar changes. Although total fructose was similar for each of the diets, the Day 7 dinner and evening snack for diets A and C contained considerably more fructose than was present in diets B and D, and in terms of glucose provided from sugars, the dinner plus evening snack on Day 7 (Table IIIA) provided 34.5 and 43 g for diets A and C, respectively, and 7.5 and 4 g from diets B and D, respectively. Further study is required to determine whether sucrose or simple sugars alter leptin secretion, independent of their effect on insulin.

Our results highlight three issues. That a single 0800 hr leptin value may not be sufficient to reveal a diet-induced change in leptin secretion; that high GI foods may have little or no effect on serum insulin in the context of a mixed meal; and that the diurnal pattern of leptin secretion may be altered by diet.

We thank Garry D. Gaddy (Institute for Research in Social Science) for his help with the statistical analysis. We are grateful to the nursing and dietary staff of the University of North Carolina General Clinical Research Center for their excellent assistance.

1. Ahima RS, Flier JS. Leptin. Annu Rev Physiol **62**:413–437, 2000.
2. Coleman RA, Herrmann TS. Nutritional regulation of leptin in humans. Diabetologia **42**:639–646, 1999.
3. Halaas J, Gajiwala K, Maffei M, Cohen S, Chait B, Rabinowitz D, Lallone R, Burley S, Friedman J. Weight-reducing effects of the plasma protein encoded by the obese gene. Science **269**:543–546, 1995.
4. Campfield L, Smith F, Guisez Y, Devos R, Burn P. Recombinant mouse ob protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science **269**:546–548, 1995.
5. Pelleymounter M, Cullen M, Baker M, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science **269**:540–543, 1995.
6. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J. Positional cloning of the mouse obese gene and its human homolog. Nature **372**:425–432, 1994.
7. Farooqi IS, Langmack G, Lawrence E, Cheetham GH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med **341**:879–884, 1999.
8. Shimabukuro M, Koyama K, Chen G, Wang M-Y, Trieu F, Lee Y, Newgard CB, Unger RH. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci U S A **94**:4637–4641, 1997.
9. Muoio DM, Dohm GL, Fiedorek FT, Tapscott EB, Coleman RA. Leptin directly alters lipid partitioning in skeletal muscle. Diabetes **46**:1360–1363, 1997.
10. Hardie L, Trayhurn P, Abramovich D, Fowler P. Circulating leptin in women: A longitudinal study in the menstrual cycle and during pregnancy. Clin Endocrinol **47**:101–106, 1997.
11. Ostlund RE, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab **81**:3909–3913, 1996.
12. Ahren B, Mansson S, Gingerich R, Havel P. Regulation of plasma leptin in mice: Influence of age, high-fat diet and fasting. Am J Physiol **273**:R113–R120, 1997.
13. Schrauwen P, van Marken Lichtenbelt W, Westerterp K, Saris W. Effect of diet composition on leptin concentration in lean subjects. Metabolism **46**:420–424, 1997.
14. Havel P, Kasim-Karakas S, Mueller W, Johnson P, Gingerich R, Stern J. Relationship of plasma leptin to plasma insulin and adiposity in

normal weight and overweight women: Effects of dietary fat content and sustained weight loss. J Clin Endocrinol Metab **81:**4406–4413, 1996.
15. Maffei M, Halaas J, Ravussin E, Pratley R, Lee G, Zhang Y, Fei H, Lallone R, Ranganathan S, Kern P, Friedman J. Leptin levels in human and rodents measurement of plasma leptin and ob RNA in obese and weigh-reduced subjects. Nat Med **1:**1155–1161, 1995.
16. Considine R, Sinha M, Heiman M, Kriauciunas A, Stephens T, Nyce M, Ohannesian J, Marco C, McKee L, Bauer T, Caro J. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med **334:**292–295, 1996.
17. Becker DJ, Ongemba LN, Brichard V, Henquin JC, Brichard SM. Diet- and diabetes-induced changes of *ob* gene expression in rat adipose tissue. FEBS Lett **371:**324–328, 1995.
18. Boden G, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. J Clin Endocrinol Metab **81:**3419–3423, 1996.
19. Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M, Caro JF. Responses of leptin to short-term fasting and refeeding in humans: A link with ketogenesis but not ketones themselves. Diabetes **45:**1511–1515, 1996.
20. Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J Clin Endocrinol Metab **82:**561–565, 1997.
21. Grinspoon SK, Askari H, Landt ML, Nathan DM, Schoenfeld DA, Hayden DL, Laposata M, Hubbard J, Klibanski A. Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. Am J Clin Nutr **66:**1352–1356, 1997.
22. Levy JR, Gyarmati J, Lesko JM, Adler RA, Stevens W. Dual regulation of leptin secretion: Intracellular energy and calcium dependence of regulated pathway. Am J Physiol Endocrinol Metab **278:**E892–E901, 2000.
23. Wang J, Liu R, Hawkins M, Barzilai N, Rosseti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature **393:**684–688, 1998.
24. Saad MF, Riad-Gabriel MG, Khan A, Sharma A, Michael R, Jinagouda SD, Boyadjian R. Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. J Clin Endocrinol Metab **83:**453–459, 1998.
25. Sinha MK, Ohannesian JP, Heiman ML, Kriauciunas A, Stephens TW, Magosin S, Marco C, Caro JF. Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. J Clin Invest **97:**1344–1347, 1996.
26. Cusin I, Sainsbury A, Doyle P, Rohner-Jeanrenaud F, Jeanrenaud F, Jeanrenaud B. The *ob* gene and insulin: A relationship leading to clues to the understanding of obesity. Diabetes **44:**1467–1470, 1995.
27. Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Stales B, Auwerx J. Transient increase in obese gene expression after food intake or insulin administration. Nature **377:**527–529, 1995.
28. Mueller WM, Gregoire F, Stanhope KL, Mobbs CV, Mizuno TM, Warden CH, Stern JS, Havel PJ. Evidence that glucose metabolism regulates leptin secretion from cultured adipocytes. Endocrinology **139:**551–558, 1998.
29. Boden G, Chen X, Kolaczynski JW, Polansky M. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest **100:**1107–1113, 1997.
30. Tuominen JA, Ebeling P, Stenman UH, Heiman ML, Stephens TW, Koivisto VA. Leptin synthesis is resistant to acute effects of insulin in insulin-dependent diabetes mellitus patients. J Clin Endocrinol Metab **82:**381–382, 1997.
31. Saad MF, Khan A, Sharma A, Michael R, Riad-Gabriel MG, Boyadjian R, Jinagouda SD, Steil GM, Kamdar V. Physiological insulinemia acutely modulates plasma leptin. Diabetes **47:**544–549, 1998.
32. Utriainen T, Malmstrom R, Makimattila S, Yki-Jarvinen H. Supraphysiological hyperinsulinemia increases plasma leptin concentrations after 4 h in normal subjects. Diabetes **45:**1364–1366, 1996.
33. Malmstrom R, Taskinen MR, Karonen SL, Yki-Jarvinen H. Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. Diabetologia **39:**993–996, 1996.
34. Vidal H, Auboeuf D, De Vos P, Staels B, Riou JP, Auwerx J, Laville M. The expression of *ob* gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. J Clin Invest **98:**251–255, 1996.
35. Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, Caro JF. Acute and chronic effects of insulin on leptin production in humans: Studies in vivo and in vitro. Diabetes **45:**699–701, 1996.
36. Halleux CM, Servais I, Reul BA, Detry R, Brichard SM. Multihormonal control of *ob* gene expression and leptin secretion from cultured human visceral adipose tissue: Increased responsiveness to glucocorticoids in obesity. J Clin Endocrinol Metab **83:**902–910, 1998.
37. Wabitsch M, Jensen PB, Blum WF, Christoffersen CT, Englaro P, Heinze E, Rascher W, Teller W, Tornqvist H, Hauner H. Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes **45:**1435–1438, 1996.
38. Havel PJ, Townsend R, Chaump L, Teff K. High-fat meals reduce 24 hour circulating leptin concentrations in women. Diabetes **48:**334–441, 1999.
39. St. Jeor ST, Stumbo PJ. Energy needs and weight maintenance in controlled feeding studies. In: Dennis BH, Ershow AG, Obarzanek E, Clevidence BA, Eds. Well-Controlled Diet Studies in Humans: A Practical Guide to Design and Management. Chicago: American Dietetic Association, pp257–262, 1999.
40. Foster-Powell K, Miller JB. International tables of glycemic index. Am J Clin Nutr **62:**871S–893S, 1995.
41. Blundell JE, Burley VJ. Satiation, satiety and the action of fiber on food intake. Int J Obesity **11:**9–25, 1987.
42. Mantzoros CS, Moschos SJ. Leptin: In search of role(s) in human physiology and pathophysiology. Clin Endocrinol **49:**551–567, 1998.
43. Keim NL, Stern JS, Havel PJ. Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. Am J Clin Nutr **68:**794–801, 1998.
44. Joannic JL, Oppert JM, Lahlou N, Basdevant A, Auboiron S, Raison J, Bornet F, Guy-Grand B. Plasma leptin and hunger ratings in healthy humans. Appetite **30:**129–138, 1998.
45. Schoeller DA, Cella LK, Sinha MK, Caro JF. Entrainment of the diurnal rhythm of plasma leptin to meal timing. J Clin Invest **100:**1882–1887, 1997.
46. Miller B. Importance of glycemic index in diabetes. Am J Clin Nutr **59:**747S–752S, 1994.
47. Sayeski PP, Kudlow JE. Glucose metabolism to glucosamine is necessary for glucose stimulation of transforming growth factor-α gene transcription. J Biol Chem **271:**15237–15243, 1996.
48. McClain D, Patterson A, Roos M, Wei X, Kudlow J. Glucose and glucosamine regulate growth factor gene expression in vascular smooth muscle cells. Proc Natl Acad Sci U S A **89:**8150–8154, 1992.
49. Daly ME, Vale C, Walker M, Littlefield A, Alberti KG, Mathews JC. Acute effects on insulin sensitivity and diurnal metabolic profiles of a high-sucrose compared with a high-starch diet. Am J Clin Nutr **67:**1186–1196, 1998.