High-Fat Diet Feeding Reduces the Diurnal Variation of Plasma Leptin Concentration in Rats

Ming C. Cha, Chieh J. Chou, and Carol N. Boozer

To investigate the response of plasma leptin and its diurnal variation to graded levels of dietary fat intake, adult (486.8 ± 10.8 g), male rats (N = 52) were fed diets containing 12%, 28%, 44%, and 60% fat for 4 weeks. The body weight gain and abdominal fat pad weight were higher ($P < .05$) in groups fed diets containing 44% and 60% fat compared with the two diets containing less fat. There were no significant differences in terms of body weight or fat pad weight between animals fed the two diets with higher fat content or between animals fed the two lower-fat diets. Twenty-four-hour energy expenditure was not different among the dietary fat groups. After 3 days on the experimental diets, plasma leptin increased ($P < .03$) in all dietary groups. The increases in leptin in animals fed 12% and 28% fat diets occurred primarily in the morning. In contrast, in groups fed the two diets containing higher fat content, leptin levels increased mainly in the afternoon. As a result, the daily variation in leptin increased ($P < .05$) in the two groups fed lower-fat diets, but decreased ($P < .04$) in animals fed the two higher-fat diets. These data demonstrate that short-term high-fat diet feeding abolished the diurnal fluctuation of plasma leptin levels, which may prevent proper leptin function and eventually contribute to the development of obesity.

LEPTIN, a protein produced by the ob gene, is synthesized and secreted mainly from adipose tissue. It has been shown in animals that leptin administration reduces food intake while maintaining energy expenditure.[1,2] Leptin acts on receptors in the hypothalamus to inhibit neuropeptide Y production; the latter stimulates food intake and decreases thermogenesis.[3] The leptin concentration increases promptly with overeating[4,5] and has been shown to be positively correlated with body lipid content.[6,7] Therefore, leptin is thought to be a humoral signal from adipose tissue that provides information to the brain about the body lipid content and energy balance, which results in adjustments of food intake and energy expenditure to maintain body weight stability.[8,9]

A diurnal variation of the circulating leptin concentration has been shown in humans. Leptin levels peak between midnight and early morning, with baseline concentrations occurring in the afternoon.[10] In rodents, ob gene expression and circulating leptin also exhibit diurnal fluctuation.[11] The timing of food intake is an independent determinant of the diurnal rhythm of leptin secretion both in man[5] and in animals.[12]

Diets high in fat have been shown to promote the development of obesity.[13] The amount of fat consumed determines the degree of body adiposity.[14,15] It has been demonstrated that a high-fat diet dramatically increases the plasma leptin level.[6,16] Presumably, an enlarged body fat mass underlies such increases in leptin. A recent study has demonstrated that lean subjects habituated to a high-fat diet had higher leptin levels as compared with low-fat diet consumers, despite a similar body mass index and body fat mass,[17] suggesting an effect of high-fat diets on the leptin level independent of body fat. Lin et al[18] also showed an acute effect of a high-fat diet on circulating leptin concentrations in rats.

However, whether the influence of dietary fat content on leptin is dose-dependent remains unknown. The magnitude of the diurnal variation of plasma leptin has been reported to correlate with the gain in total body fat,[19,20] but it is not clear whether high-fat diets influence the daily fluctuation of the circulating leptin level. The present study was therefore designed to investigate the effect of graded quantities of dietary fat on the circulating leptin concentration and its diurnal variation. The potential relationship involving dietary fat, leptin, and body weight gain was also examined.

MATERIALS AND METHODS

Animals and Diets

Fifty-two adult male Sprague-Dawley rats (486.8 ± 10.8 g) were obtained from the breeding colony at the Animal Care Facility of St. Luke's-Roosevelt Hospital Center. Rats were housed in separate wire-mesh cages in an isothermal (23° ± 1°C) light cycle–controlled (7 AM to 7 PM light) environment and were fed ad libitum on a commercial rat chow (Lab Diet; PMI Nutrition International, St Louis, MO) before the feeding trial. The rats were then randomly assigned to 4 dietary groups of 13 rats each and switched to defined diets containing either 12%, 28%, 44%, or 60% energy as fat ad libitum for 4 weeks (Table 1). The ingredients of the experimental diets were purchased from Teklad Premier Laboratory Diets (Madison, WI) and the diets were mixed in our laboratory. Body weight and food intake were recorded 3 times per week. Blood samples were collected by tail bleeding from 8 to 10 AM and 2 to 4 PM 3 days before and 3 days after the first day of feeding the experiment diets. At the end of week 4 between 9 AM and 12 noon, the animals were lightly anesthetized using carbon dioxide gas and a blood sample was collected by heart puncture. The rats were then killed by decapitation. Epididymal and retroperitoneal fat pads were excised bilaterally and weighed, with data presented as the average of the two sides.

Energy Expenditure

Twenty-four-hour energy expenditure was determined using our small-animal respiratory system as previously described.[1] Briefly, this is a computer-driven system that allows simultaneous testing by serial sampling of up to 6 animals housed in individual sealed Plexiglas (Rohm & Haas, Philadelphia, PA) metabolic chambers (School of Engineering, Columbia University). The chambers are maintained in a separate controlled-environment room with constant temperature and humidity and supplied with first-pass fresh air. Air sampled from the

From the Obesity Research Center, St. Luke's-Roosevelt Hospital, and the Institute of Human Nutrition, Department of Medicine, Columbia University, New York, NY.

Submitted June 8, 1999; accepted September 7, 1999.

Supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants No. DK49853 and P30DK26687.

Address reprint requests to Carol N. Boozer, DSc, WH 1029, Obesity Research Center, St. Luke's–Roosevelt Hospital Center, 1111 Amsterdam Ave, New York, NY 10025.

0026-0495/00/4904-0018$10.00/0

Table 1. Composition of the Experimental Diets

Component (g/100 g)	Fat Content			
	12%	28%	44%	60%
Casein	15	15	15	15
Fat	5	13	23	36
Cornstarch	50	44	37	28
Sucrose	20	18	15	11
Cellulose	5	5	5	5
AIN-93M mineral mix	3.5	3.5	3.5	3.5
AIN-93M vitamin mix	1	1	1	1
L-Cystine	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25
tert-Butylhydroquinone	0.0008	0.0008	0.0008	0.0008

NOTE. Values are based on the formulation of the AIN-93M rodent diet.

Abbreviation: AIN, American Institute of Nutrition.

chambers is analyzed for oxygen (Magnos IV oxygen analyzer) and carbon dioxide (Uras 3G carbon dioxide analyzer; both Hartmann Braun, Frankfurt, Germany) for 23 hours. From these values, the average energy expenditure per minute is calculated on-line using standard formulae[21] and extrapolated to 24 hours. Rats were tested 1 or 2 days prior to death.

Leptin and Insulin Assays

Leptin and insulin assays were performed in duplicate by the Hormone/Metabolite Core Laboratory of the New York Obesity Research Center using radioimmunoassay kits (Linco Research, St Charles, MO). For the leptin assay, the sensitivity is 0.5 ng/mL, the limit of linearity 50 ng/mL (100-μl sample size), and the coefficient of variation (CV) 8.8%. For the insulin assay, the sensitivity is 0.1 ng/mL, the limit of linearity 10 ng/mL, (100-μl sample size), and the CV 5.3%.

Statistical Methods

We assigned 13 rats per dietary fat group, following sample size analysis showing that 12.9 rats per group would be required to detect a difference in daily leptin variation of 2 ng, with a standard deviation of 3 ng and power at 80%.

The data are presented as the mean ± SEM. The main effect of dietary fat was determined by 1-way ANOVA using the SPSS general linear model program (SPSS, Chicago, IL). Fisher's protected least-significant difference test was used to determine the difference between means.

RESULTS

The body weight gain was higher ($P < .01$) in groups fed the diets with 48% (84.6 ± 8.3 g) or 60% (83.8 ± 7.9 g) of energy as fat compared with animals fed 12% fat (38.3 ± 4.4 g) and 28% fat (52.3 ± 6.0 g) diets (Fig 1). There was no difference in body weight gain between the groups fed the two higher-fat diets or between the groups fed the two lower-fat diets. Similar results were observed for the epididymal and retroperitoneal fat pad weight. Energy expenditure was not different among the dietary groups. As expected, the respiratory quotient differed among dietary groups, with the highest values in the 12% fat group (Table 2). Energy intake was higher ($P < .05$) during the first 3 days on the experimental diet in the animals fed the 60% fat diet (354.5 ± 19.4 kcal) compared with groups fed 12% fat (301.3 ± 15.2 kcal) and 28% fat (299.4 ± 15.5 kcal) diets (Fig 2). However, there was no difference regarding energy intake between the groups fed the two higher-fat diets or the two groups fed the lower-fat diets. Similarly, total energy intake over the 4-week test was only different ($P < .05$) between groups fed the two higher levels of fat (2,866.2 ± 68.2 *v* 2,800.4 ± 84.5 kcal for the 44% and 60% fat groups, respectively) as compared with the two lower-fat diets (2,460.7 ± 68.1 *v* 2,585.5 ± 74.3 kcal for the 12% and 28% fat groups).

Plasma leptin concentrations at 3 days before the diet change were not different among the groups either in the morning or in the afternoon (Fig 3). However, after 3 days of the experimental diets, morning leptin levels were 20% higher ($P < .05$) in animals fed the 48% fat diet (20.3 ± 1.1 ng/mL) compared with rats fed the 12% (16.7 ± 1.4 ng/mL) or 28% (16.2 ± 1.4 ng/mL) fat diets. There were no differences in morning leptin levels between the 12% and 28% fat dietary groups or between the 44% and 60% (19.0 ± 1.2 ng/mL) fat groups. In contrast to the morning leptin, afternoon leptin concentrations were increased about 40% ($P < .01$) in rats fed the 44% fat (19.7 ± 1.8 ng/mL) and 60% fat (20.5 ± 1.4 ng/mL) diets as compared with animals fed the 12% fat (12.1 ± 1.4 ng/mL) or 28% fat (12.5 ± 1.5 ng/mL) diets.

The daily fluctuations in leptin (differences between morning and afternoon levels) were not different among the groups before the dietary treatment (3.24 ± 0.63, 2.11 ± 0.31, 2.10 ± 0.39, and 2.52 ± 0.49 ng/mL for 12%, 28%, 44%, and 60% fat dietary groups, respectively; Fig 4). However, after consumption of the experimental diets for 3 days, the daily changes in plasma leptin levels from morning to afternoon were increased ($P < .02$) in the 12% fat (4.58 ± 0.83 ng/mL) and 28% fat (3.94 ± 0.67 ng/mL) dietary groups and decreased ($P < .05$) in the 44% fat (0.63 ± 1.81 ng/mL) and 60% fat (−1.46 ± 1.36 ng/mL) dietary groups. When data for the leptin daily variation were further analyzed controlling (by covariate analysis) for the effects of energy intake for the first 3 days and body weight, the *P* value for the effect of dietary fat on the leptin daily variation changed from $P = .005$ to $P = .03$ after controlling for the effect of 3-day energy intake and to $P = .006$ after controlling for the effect of body weight at the end of 1-week feeding.

Leptin levels at death were higher ($P < .05$) in the 44% fat (36.9 ± 3.8 ng/mL) and 60% fat (38.7 ± 6.3 ng/mL) groups as compared with 12% fat (23.5 ± 3.6 ng/mL) diet group (Fig 5).

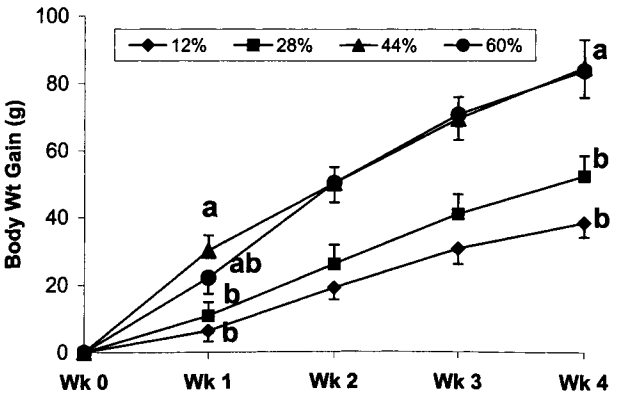

Fig 1. Body weight gain in rats fed diets varying in fat content for 4 weeks. Values are the mean ± SEM (n = 13). Means with different letter at the same week are significantly different.

Table 2. Body Weight, Fat Pad Weight, Respiratory Quotient, and Energy Expenditure in Rats Fed Diets Containing Various Quantities of Fat

Parameter	Fat Content			
	12%	28%	44%	60%
Body weight (g)				
Initial	488.9 ± 11.7	486.2 ± 11.5	488.2 ± 10.3	483.7 ± 9.6
At death	527.3 ± 13.7[a]	538.4 ± 16.1[a]	572.7 ± 17.1[b]	567.5 ± 15.7[b]
Fat pad weight (g)				
Epididymal	5.37 ± 0.47[a]	5.77 ± 0.45[a]	7.73 ± 0.57[b]	8.22 ± 0.77[b]
Retroperitoneal	7.40 ± 0.55[a]	8.16 ± 0.58[a]	10.62 ± 0.60[b]	10.80 ± 0.61[b]
Respiratory quotient	0.92 ± 0.01[a]	0.88 ± 0.01[b]	0.84 ± 0.01[c]	0.81 ± 0.02[d]
Energy expenditure (cal/min)	69.3 ± 2.9	71.8 ± 2.9	72.1 ± 2.3	74.6 ± 2.7

NOTE. Values are the mean ± SEM. For each parameter, means with different superscripts are significantly different.

Differences in leptin concentrations at death were not significant between the 44% fat (36.9 ± 3.8 ng/mL) and 60% fat dietary groups or between the two groups fed less fat.

Plasma insulin concentrations were not different among the dietary groups either before dietary treatment or at death (Table 3). After 3 days on the experimental diets, morning insulin levels were lower ($P < .03$) in the 3 groups fed higher levels of fat compared with rats fed the 12% fat diet or compared with morning insulin levels before the dietary treatment or at death. Afternoon insulin levels did not differ among the groups 3 days after dietary treatment.

DISCUSSION

Diets that are high in fat promote the development of obesity. Leptin has been proposed to act as a feedback signal to regulate food intake and energy expenditure and therefore to maintain body weight stability. However, the role of leptin in the development of obesity in response to a high-fat diet remains to be elucidated. The present study demonstrates for the first time that high-fat diets abolished the diurnal variation of plasma leptin concentrations. The lack of a daily fluctuation of the circulating leptin concentration may impair leptin action and eventually lead to obesity.

It has been shown that consumption of a high-fat diet promotes greater weight gain and excess body fat deposition. Doucet et al[22] investigated the relationship between dietary fat content and body adiposity in adult men. Significant positive correlations were found between the percentage of dietary energy as fat and body fatness. We have previously reported that in rats fed isocalorically on diets containing 24%, 36%, or 48% energy as fat, total body fat and adipose depot weight increase in proportion to the level of fat in the diet, despite similar body weight.[14] In the present study, the gain in body weight was not in proportion to the fat content in the group fed the highest level of dietary fat. The fluid nature of the 60% fat diet may make it difficult for the rats to eat. As a result, animals in this dietary group did not consume more energy or gain more weight as

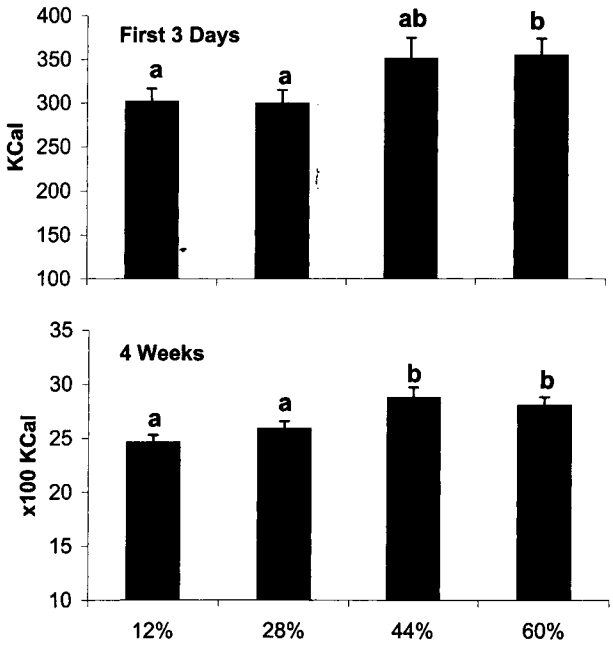

Fig 2. Energy intake for the first 3 days and total 4-week feeding trial of rats fed diets varying in fat content. Values are the mean ± SEM (n = 13). Means with different letters are significantly different.

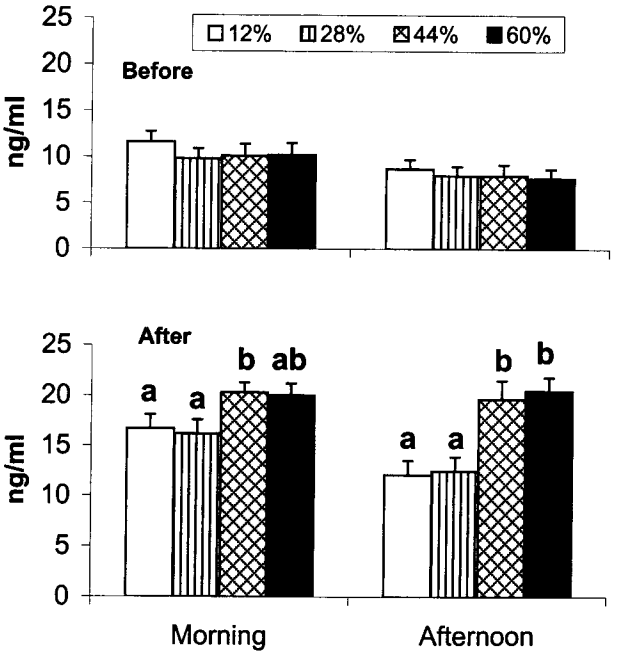

Fig 3. Plasma leptin in rats fed diets varying in fat content 3 days before dietary treatment and after 3 days' consumption of the experimental diets. Values are the mean ± SEM (n = 12-13). Means with different letters are significantly different.

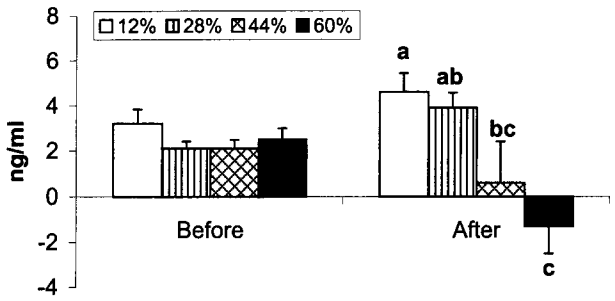

Fig 4. Magnitude of variation in plasma leptin levels from morning to afternoon 3 days before the dietary treatment and 3 days after consuming the experimental diets. Values are the mean ± SEM (n = 12-13).

compared with rats fed the 44% fat diet, although the fat pad weight was higher, consistent with our previous findings.[14]

Food intake was influenced by the fat content in the diet. Energy intake for the first 3 days of the experimental diet was higher in the two high-fat diet groups versus the two groups fed lower-fat diets. These differences in energy intake remained throughout the feeding period. However, energy expenditure was not different among the dietary groups. An equal energy expenditure but higher energy intake in the high-fat diet animals compared with low-fat diet groups explains the difference in body weight gain.

The diurnal rhythm of the circulating leptin level has been shown to be determined by the timing of food intake.[5,12] Rats usually eat in the dark. We thus hypothesized that with a 12-hour light/dark cycle (7 AM to 7 PM light), leptin levels in our rats would peak between 8 and 10 AM and decrease to the nonfed baseline level by 2 to 4 PM. Our results support such a hypothesis.

High-fat diets have been shown to increase leptin levels. Frederich et al[6] fed diets containing 21% (wt/wt) fat to mice for 12 weeks and found that plasma leptin increased greatly. However, increased body adiposity may account for the elevated leptin levels. Similarly, in women consuming a diet with 50% energy as fat for 3 weeks, leptin levels were significantly increased.[16] Again, changes in body composition may be an explanation. Recently, increased leptin levels have been demonstrated in rats fed a diet with 56% energy as fat for 2 days,[18] suggesting an effect of a high-fat diet on the leptin concentration independent of body lipid stores. In the present study, high-fat diets increased leptin levels both in the morning and in the afternoon. Since body composition is probably not significantly changed within 3 days, our results demonstrate a hyperleptinemia effect of overeating associated with high-fat diet consumption.

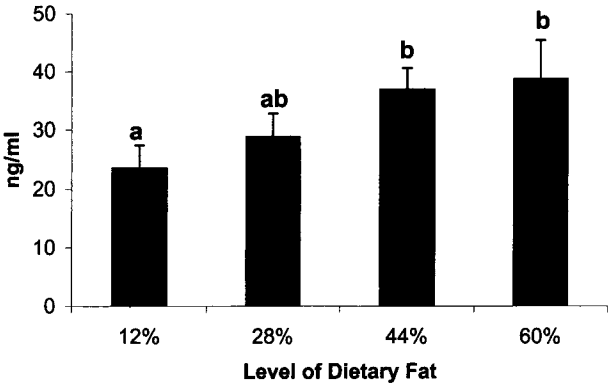

Fig 5. Plasma leptin at death in rats fed diets varying in fat content for 4 weeks. Values are the mean ± SEM (n = 11-13).

Table 3. Plasma Insulin in Rats Fed Diets With Various Quantities of Fat Before and After Starting the Dietary Regimen and at Death

Plasma Insulin (ng/mL)	Fat Content			
	12%	28%	44%	60%
3 Days before				
Morning	2.22 ± 0.34	2.27 ± 0.33	2.26 ± 0.28	1.90 ± 0.33
Afternoon	1.63 ± 0.21	1.61 ± 0.30	1.76 ± 0.36	1.64 ± 0.28
3 Days after				
Morning	2.39 ± 0.55[a]	1.09 ± 0.22[b]	1.36 ± 0.29[b]	1.00 ± 0.23[b]
Afternoon	2.07 ± 0.46	1.59 ± 0.29	2.69 ± 0.52	1.76 ± 0.33
At death	6.88 ± 1.04	5.28 ± 0.49	6.19 ± 0.84	6.45 ± 0.69

NOTE. Values are the mean ± SEM. For each parameter, means with different superscripts are significantly different.

The present data further show that leptin levels increased more in the afternoon than in the morning in response to high-fat diet feeding. In contrast, in animals fed low-fat diets, the major increase in leptin was observed in the morning. As a consequence, the diurnal variation of leptin levels strikingly decreased in the two high-fat diet groups, while it increased in the low-fat diet animals. One possible explanation may be that the high-fat diet changed the eating pattern in those animals. Rats fed the high-fat diet might eat in both the dark and the light periods. However, this was not observed. In addition, the amount of food eaten (in grams) is not different among the dietary groups and is even lower numerically in the high-fat diet groups (data not shown). It does not appear that a longer eating period or a shift in the timing of eating is associated with the rats fed the high-fat diets. It has been reported that the average circadian amplitude of plasma leptin levels between the acrophase and nadir is lower in obese compared with lean subjects.[10,20] Gains in body fat are inversely related to the nocturnal increase (peak) in serum leptin.[19] In considering these findings, the present results suggest that the decreased diurnal fluctuation of leptin levels may be related to an impaired action of leptin as a feedback signal and contribute to the body fat accumulation in the high-fat diet groups.

Plasma insulin has been reported as positively correlated with leptin levels.[23] In the present study, morning insulin levels decreased in groups fed the 3 higher-fat diets. The exact mechanism remains to be determined. The decreased carbohydrate content in the high-fat diet compared with the rodent chow may reduce glucose-induced insulin secretion. It has been reported that leptin inhibits insulin secretion.[24] Increased leptin levels after 3 days' consumption of the purified diets may blunt insulin secretion, especially when the percentage of carbohydrate in the diet is decreased. Plasma insulin increased after 4 weeks of the feeding trial across dietary groups. This could be due to the gains in body weight and fat, suggesting relative insulin resistance in those animals. However, no difference regarding the insulin concentration was observed among the

dietary groups, despite the higher body weight and fat gains in high-fat diet groups. The combined effect of a higher leptin level and lower glucose supply in the high-fat diet groups may be an explanation.

In summary, high-fat diet feeding associated with overeating increased plasma leptin and decreased the diurnal variation of the leptin concentration. The reduced diurnal fluctuation of circulating leptin may obstruct leptin's action as a feedback signal and lead to the development of obesity.

ACKNOWLEDGMENT

The technical support of Lina Basilio, Yim Dam, and Kangping Chen is greatly appreciated.

REFERENCES

1. Halaas JL, Boozer CN, Blair-West J, et al: Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci USA 94:8878-8883, 1997

2. Campfield LA, Smith FJ, Guisez Y, et al: Recombinant mouse ob protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546-549, 1995

3. Meier CA: Leptin secretion and action: An update. Eur J Endocrinol 134:543-544, 1996

4. Kolaczynski JW, Ohannesian JP, Considine RV, et al: Response of leptin to short-term and prolonged overfeeding in humans. J Clin Endocrinol Metab 81:4162-4165, 1996

5. Taylor AE, Hubbard J, Anderson EJ: Impact of binge eating on metabolic and leptin dynamics in normal young women. J Clin Endocrinol Metab 84:428-434, 1999

6. Frederich RC, Hamann A, Anderson S, et al: Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. Nat Med 1:1311-1314, 1995

7. Considine RV, Sinha MK, Heiman ML, et al: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292-295, 1995

8. Caro JF, Sinha MK, Kolaczynski JW, et al: Leptin: The tale of an obesity gene. Diabetes 45:1455-1462, 1996

9. Pelleymounter MA, Cullen MJ, Bak MB, et al: Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540-543, 1995

10. Sinha MK, Ohannesian JP, Heiman ML, et al: Noctural rise of leptin in lean, obese, and non–insulin-dependent diabetes mellitus subjects. J Clin Invest 97:1344-1347, 1996

11. Saladin R, De Vos P, Guerre-Millo M, et al: Transient increase in obese gene expression after food intake or insulin administration. Nature 377:527-529, 1995

12. Ahima RS, Prabakaran D, Flier JS: Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. J Clin Invest 101:1020-1027, 1998

13. Curb JD, Marcus EB: Body fat and obesity in Japanese Americans. Am J Clin Nutr 53:1552S-1555S, 1991 (suppl 6)

14. Boozer CN, Schoenbach G, Atkinson RL: Dietary fat and adiposity: A dose-response relationship in adult male rats fed isocalorically. Am J Physiol 268:E546-E550, 1995

15. Toubro S, Astrup A: Ad libitum low-fat, high-carbohydrate diet versus calorie-counting for weight maintenance after major weight loss in obese patients. BMJ 314:29-34, 1997

16. Lovejoy JC, Windhauser MM, Rood JC, et al: Effect of a controlled high-fat versus low-fat diet on insulin sensitivity and leptin levels in African-American and Caucasian women. Metabolism 47:1520-1524, 1998

17. Cooling J, Barth J, Blundell J: The high-fat phenotype: Is leptin involved in the adaptive response to a high fat (high energy) diet? Int J Obes Relat Metab Disord 22:1132-1135, 1998

18. Lin X, Chavez MR, Bruch RC, et al: The effects of a high fat diet on leptin mRNA, serum leptin and the response to leptin are not altered in a rat strain susceptible to high fat diet–induced obesity. J Nutr 128:1606-1613, 1998

19. Matkovic V, Ilich JZ, Badenhop NE, et al: Gain in body fat is inversely related to the nocturnal rise in serum leptin level in young females. J Clin Endocrinol Metab 82:1368-1372, 1997

20. Saad MF, Riad-Gabriel MG, Khan A, et al: Diurnal and ultradian rhythmicity of plasma leptin: Effects of gender and adiposity. J Clin Endocrinol Metab 83:453-459, 1998

21. Brouwer E: Report of sub-committee on constants and factors. Appendix III, in McLean JA, Tobin G (eds): Animal and Human Calorimetry. Cambridge, UK, Cambridge University Press, 1987

22. Doucet E, Almeras N, White MD, et al: Dietary fat composition and human adiposity. Eur J Clin Nutr 52:2-6, 1998

23. Widjaja A, Stratton IM, Horn R, et al: Plasma leptin, obesity, and plasma insulin in type 2 diabetic subjects. J Clin Endocrinol Metab 82:654-657, 1997

24. Poitout V, Rouault C, Guerre-Millo M, et al: Inhibition of insulin secretion by leptin in normal rodent islets of Langerhans. Endocrinology 139:822-826, 1998