Increased Plasma Leptin Levels Are Associated With Fat Accumulation in Japanese Americans

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Although the hormone leptin seems to play a role in ensuring the maintenance of adequate energy stores and thereby protects against starvation, its role in the regulation of body weight and adiposity under normal circumstances is unclear. Overweight individuals have markedly elevated circulating leptin levels, suggesting that leptin's effect on food intake and thermogenesis is diminished or absent in obesity. Recent evidence, though, indicates that weight gain in Pima Indians is associated with relatively decreased levels of the hormone. Because it is important to understand whether a deficiency in circulating leptin contributes to the development of obesity, we sought to determine whether there is a relationship between leptin levels and subsequent changes in adiposity in a more typical population. We compared baseline plasma leptin concentrations to changes over 5 years in body weight, BMI, and computed tomography–determined total fat in 492 second- and third-generation Japanese Americans. Subjects were of 100% Japanese ancestry; male subjects had a mean BMI at baseline of 25.4 kg/m^2 and a mean age of 54 years; female subjects had a mean BMI of 23.1 kg/m^2 and a mean age of 53 years. Changes in weight (men: $r = 0.17$, $P < 0.05$; women: $r = 0.20$, $P < 0.05$), BMI (men: $r = 0.17$, $P < 0.05$; women: $r = 0.18$, $P < 0.05$), and total fat (men: $r = 0.19$, $P < 0.05$; women: $r = 0.20$, $P < 0.01$) were positively correlated with baseline leptin levels adjusted for baseline adiposity, fasting insulin, and age. In Japanese Americans, then, relatively increased leptin levels are associated with greater subsequent gains in weight and adiposity. We concluded that in this population, fat accumulation is associated not with leptin deficiency but possibly with leptin resistance and is preceded by increased leptin levels.
***Diabetes* 47:239–243, 1998**

Circulating levels of the fat-derived hormone leptin increase with adiposity (1–5). Because leptin is transported into the central nervous system (6), it provides a means by which the brain can monitor fat stores. Ample evidence suggests that leptin feedback to the brain plays an important role in maintaining adequate energy reserves. Leptin levels fall in response to decreased adiposity and short-term fasting (2,3,7,8), and decreased leptin levels prompt increased appetite and reduced thermogenesis (4,5). Recombinant leptin administered to genetically leptin-deficient *ob/ob* mice markedly decreases feeding and suppresses the obesity these animals otherwise develop (4,5).

Although leptin seems to play an important role in protecting against depletion of fat stores and starvation, its effects on feeding, metabolism, and weight regulation are less certain when food supplies are not limited and energy stores are adequate. Leptin's role in the regulation of energy balance under these permissive circumstances is of particular interest because of the high and increasing prevalence of obesity and its associated morbidity (9). The elevated leptin levels that always accompany increased adiposity, together with the apparent rarity of mutations that impair the functioning of the hormone in overweight individuals, suggest that one cause of obesity may be resistance to the actions of leptin (1–3,10). Alternatively, prevention of obesity may not be an important physiological role of leptin, and its effects on satiety and thermogenesis may normally plateau early in the course of excessive fat accumulation (5). To date, the lack of availability of leptin for use in humans has prevented direct testing of the significance of leptin resistance in obesity.

Results recently reported by Ravussin et al. (11) have suggested that at least in some cases, relatively low plasma leptin levels rather than leptin resistance may underlie excessive fat accumulation. They compared baseline leptin levels in a group of 19 Pima Indians selected for weight gain >3.5 kg/year to levels in a group of weight-stable Pima Indians and found that the subjects who gained weight had significantly lower baseline plasma leptin levels after adjusting for adiposity. Pima Indians are especially prone to obesity; the mean baseline BMI of the weight-gaining subjects was 36.5 kg/m^2. To ascertain whether plasma leptin levels are linked to subsequent weight gain in a population less prone to obesity, we studied the relationship between baseline leptin levels and subsequent changes in weight and adiposity over a 5-year period in 492 second- and third-generation Japanese Americans. In contrast with the findings of Ravussin et al., our results suggested that, in Japanese Americans, relatively increased leptin levels are predictive of subsequent increases in weight and body fat.

RESEARCH DESIGN AND METHODS

Study population. Subjects were drawn from the Japanese-American Community Diabetes Study (JACDS). All individuals with 5-year follow-up weight and computed tomography (CT) adiposity data were included. Selection and recruitment of volunteers and determination of fasting serum glucose and plasma insulin levels and other metabolic parameters have been described previously (12,13). The JACDS study population is typical of the Japanese-American population of King County, Washington. CT quantification of regional fat distribution was performed using the method described by Shuman et al. (14), and has been described in con-

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Received for publication 18 June 1997 and accepted in revised form 15 October 1997.

CT, computed tomography.

junction with this population elsewhere (13,15). Briefly, 10-mm slices of the thorax on inspiration at the level of the nipples, the abdomen at the level of the umbilicus, and the mid-thigh at a level halfway between the greater trochanter and the superior margin of the patella were analyzed for cross-sectional area of adipose tissue (cm^2), defined as ranging from –250 to –50 Hounsfield units. Total fat area was taken as the sum of the subcutaneous adipose areas from the thorax, abdomen, and mid-thigh and the intra-abdominal adipose area, measured using the transversalis fascia as the outer boundary. All study procedures were approved by the University of Washington Human Subjects Review Committee.

Determination of leptin levels. Plasma leptin levels were determined in duplicate using a radioimmunoassay kit (Linco Research, St. Charles, MO) described in detail by Zhongmin et al. (16). This assay measures total (bound plus free) leptin in the circulation. The limits of sensitivity and linearity for the assay are 0.5 and 100 ng/ml, respectively. The intra-assay coefficient of variation is reported by the manufacturer to be 3–9% and in our hands was 4.8%. The interassay coefficient of variation, reported to be 3–7%, was 7.2% for a 2.4 ng/ml sample and 5.6% for a 14.8 ng/ml sample. Assays were performed according to the manufacturer's instructions. Plasma from fasting morning blood draws (7:30–8:30 A.M.) was stored at –80°C and thawed just before use.

Statistical analysis. Analysis of variance models were used to test the significance of means and test for linear trends across groups. The χ^2 test was used to test for differences among categorical variables (17). Linear regression analysis was used to assess the correlation between leptin and measures of weight gain and to adjust correlations for baseline factors (18). Because of the positive skewness of the leptin variable, a 95% CI for correlations, partial correlations, and regression coefficients for linear trends in analysis of variance models was computed using nonparametric bootstrap methods to confirm significant results (not shown) (19). Data are expressed as means ± SE.

RESULTS

Of 658 subjects who underwent baseline evaluation, 574 (87%) were reexamined at 5 years; 70 were lost to follow-up and 14 died. Data collected on 82 subjects were insufficient for analysis. Baseline characteristics of the remaining 492 Japanese-American subjects are shown in Table 1. Cross-sectional fat areas from CT scans of the thigh, abdomen, and thorax were summed to obtain total CT fat area, a measure of adiposity that we have found correlates with leptin levels more closely than does BMI (15). Although leptin levels are significantly higher ($P < 0.05$) for women with NIDDM than for those without it, this difference disappears after adjusting for total CT fat, which is also significantly higher. This agrees with our previous determination that there is no correlation between the diagnosis of impaired glucose tolerance or NIDDM and leptin levels in Japanese Americans (15).

To elucidate the relationship between baseline plasma leptin levels and subsequent changes in weight and fat content, we computed correlation coefficients between baseline leptin levels and changes over 5 years in body weight, BMI, and total CT fat area (Table 2). Also presented are partial correlation coefficients between baseline leptin concentration and changes in weight, BMI, and total CT fat area, adjusting for baseline measurements of weight, BMI, and total CT fat area, respectively, and for age and fasting insulin, two potentially confounding factors (13). After these adjustments, higher baseline leptin levels correlated significantly with greater gains in all three measures of weight and adiposity in nondiabetic men and women. There was also a significant correlation before the adjustments in women. The number of subjects with NIDDM was smaller, and no significant relationship between baseline leptin levels and changes in adiposity was detected in this subset.

In a second analysis, Japanese-American subjects were divided into quartiles based on 5-year change in adiposity as measured by change in total CT fat area. Leptin levels were adjusted as in Table 2 and also, because of the inclusion of subjects with NIDDM, for baseline diabetes status. Mean leptin levels after adjustments are shown for each of the quartiles in Table 3. Leptin levels generally increased from the first quartile, comprised of men or women whose adiposity decreased, to the fourth quartile, comprised of the subjects with the greatest increase in fat ($P < 0.01$ for differences among the means and $P < 0.005$ for linear trend for both men and women). Japanese-American women had significantly higher adjusted leptin levels than their male counterparts ($P < 1 \times 10^{-7}$), consistent with results in other populations (6,20–22).

Baseline leptin levels for the first and fourth quartiles of change in adiposity (as measured by change in total CT fat area) versus baseline total CT fat area are plotted in Fig. 1. As expected, leptin increased with adiposity. The regression lines for the first and fourth quartiles show that, at any given level of body fat content above a minimum total CT fat area of ~200 cm^2, the plasma leptin level was greater on average in the subjects belonging to the fourth quartile and that this difference increased with fat content. The regression lines intersect when total CT fat is ~150–200 cm^2, so that the relationship between leptin concentration and subsequent change in adiposity did not seem to hold for those subjects with the least baseline fat content.

DISCUSSION

In summary, we found that Japanese-American individuals with relatively higher plasma leptin levels for their degree of

TABLE 1
Baseline characteristics of 492 Japanese-American men and women by NIDDM status at baseline

	Men			Women		
	Nondiabetic at baseline	NIDDM at baseline	*P*	Nondiabetic at baseline	NIDDM at baseline	*P*
n	235	55		174	28	
Age (years)	51.8 ± 0.8	61.0 ± 0.7	<0.0001	52.0 ± 0.9	62.1 ± 1.3	<0.0001
Weight (kg)	71.1 ± 0.7	71.3 ± 1.3	0.92	54.6 ± 0.6	58.0 ± 1.9	0.041
BMI (kg/m^2)	25.3 ± 0.2	25.7 ± 0.4	0.30	22.8 ± 0.2	24.9 ± 0.7	0.0012
Total CT fat (cm^2)	415 ± 11	440 ± 18	0.24	524 ± 16	681 ± 40	0.0002
Leptin (ng/ml)	4.1 ± 0.2	3.8 ± 0.2	0.30	11.7 ± 0.6	15.7 ± 1.7	0.0098
Fasting insulin (pmol/l)	76.3 ± 2.8	96.2 ± 8.3	0.025	82.6 ± 3.2	134.4 ± 12.4	0.0003
Fasting glucose (mmol/l)	5.38 ± 0.04	9.23 ± 0.45	<0.0001	5.03 ± 0.04	8.00 ± 0.58	<0.0001

Data are means ± SE. *P* value for comparison of means derived using the two-sample *t* test.

TABLE 2
Correlation coefficients and partial correlation coefficients between 5-year change in measures of weight or adiposity and baseline plasma leptin for Japanese-American men and women

	Nondiabetic at baseline		NIDDM at baseline	
	r	Adjusted r	r	Adjusted r
Men (n = 235 nondiabetic, 55 diabetic)				
Weight change	0.10	0.17*	–0.04	0.14
BMI change	0.10	0.17*	–0.04	0.08
Total CT fat area change	0.02	0.19*	–0.04	0.11
Women (n = 174 nondiabetic, 28 diabetic)				
Weight change	0.18*	0.20*	–0.09	–0.15
BMI change	0.19*	0.18*	–0.09	–0.11
Total CT fat area change	0.22†	0.20†	0.09	0.15

Partial correlation coefficients for change in weight, BMI, and total CT fat area are adjusted for baseline weight, BMI, and total CT fat area, respectively, and also for baseline fasting insulin and age. *P < 0.05; †P < 0.01.

adiposity or weight experienced greater increases in weight and fat content over 5 years than those with lower levels. Our results identified increased plasma leptin concentration as a predictor of increased adiposity and weight gain in this population.

These results differ from those of Ravussin et al. (11), who found that weight gain in Pima Indians was associated with relatively decreased leptin levels. Pima Indians, unlike Japanese Americans, are unusually prone to obesity and insulin resistance. The mean BMI of the Japanese-American subjects in our study was 24.4 kg/m^2, whereas the mean BMI of the Pima Indian subjects in the study of Ravussin et al. was 35.3 kg/m^2. The Pima Indians are also atypical in that their adiposity-adjusted plasma leptin concentrations did not vary between men and women. In the population studied here and in others (6,20–22), leptin levels are significantly greater in women than in men. Finally, in addition to analyzing a group of people more representative of the general population, our study used a substantially greater number of subjects than did that of Ravussin et al.: 492 vs. 36.

Leptin is secreted into the bloodstream by white adipose tissue, and circulating levels generally reflect fat stores (4,5). Dif-

TABLE 3
Baseline characteristics of 492 Japanese Americans by quartile of total CT fat change over a 5-year follow-up

	1st quartile	2nd quartile	3rd quartile	4th quartile	P
Men (n = 290)					
Change in fat (cm^2)*	ΔCT <–29	–29 < ΔCT < 26	26 < ΔCT < 80	ΔCT >80	
Age (years)	53.8 ± 1.3	55.9 ± 1.3	54.9 ± 1.3	49.6 ± 1.4	0.0046
Total CT fat (cm^2)	460 ± 17	423 ± 19	400 ± 18	397 ± 23	0.083 L: 0.015
Fasting insulin (pmol/l)	92.4 ± 6.9	76.4 ± 5.2	77.4 ± 5.0	73.1 ± 4.2	0.056 L: 0.019
% diabetic at baseline	26.0	18.1	20.8	11.0	0.13
Leptin (ng/ml)†	3.5 ± 0.2	3.9 ± 0.2	4.3 ± 0.2	4.4 ± 0.2	0.0072 L: 0.0006
Women (n = 202)					
Change in fat (cm^2)*	ΔCT <–5	-5 < ΔCT < 57	57 <ΔCT < 136	ΔCT >136	
Age (years)	55.2 ± 1.7	56.1 ± 1.6	52.5 ± 1.7	49.6 ± 1.7	0.026
Total CT fat (cm^2)	513 ± 28	598 ± 32	507 ± 26	564 ± 32	0.096 L: 0.65
Fasting insulin (pmol/l)	84.6 ± 7.3	96.0 ± 6.8	79.5 ± 4.8	99.0 ± 8.4	0.15 L: 0.40
% diabetic at baseline	14.0	17.6	11.8	12.0	0.81
Leptin (ng/ml)†	11.0 ± 0.5	11.4 ± 0.7	13.6 ± 0.7	13.0 ± 0.6	0.0092 L: 0.0042

Data are means ± SE. Differences between means among groups and linear trends (L) across groups were tested with analysis of variance; differences between percentages were tested with a χ^2 test. *Range of change in total CT fat area (ΔCT) within quartile. †Means and P value for leptin are adjusted for baseline total CT fat, baseline fasting insulin, baseline diabetes status, and age.

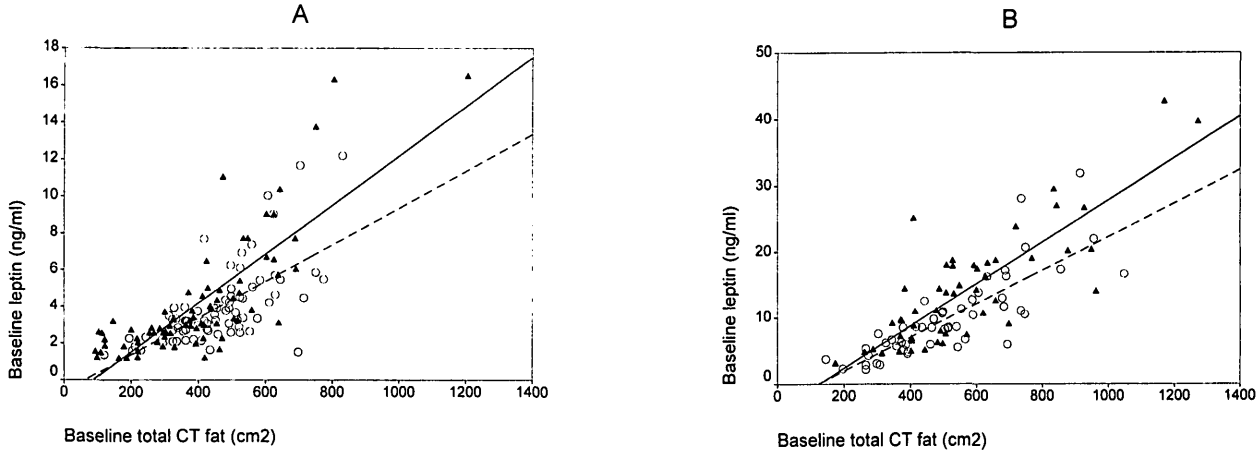

FIG. 1. Relationship of baseline plasma leptin concentration to baseline CT-determined total fat for the first and fourth quartiles of change in total fat in men (*A*) and women (*B*). The solid lines are the regression lines for the fourth quartiles, members of which (▲) had the greatest 5-year increase in adiposity (men: $r = 0.81$, $P < 0.0001$; women: $r = 0.82$, $P < 0.0001$); the dashed lines are for the first quartiles (○; men: $r = 0.65$, $P < 0.0001$; women: $r = 0.81$, $P < 0.0001$).

ferences in plasma concentrations among individuals with the same degree of adiposity but different propensities to gain fat must be accounted for by either differences in the rate of leptin synthesis or secretion by adipocytes or differences in leptin removal from the blood. Which of these mediates the variation in plasma leptin levels observed here is unknown. Although insulin increases leptin synthesis (23), we found plasma leptin concentration and fat accumulation to be correlated after adjusting for plasma fasting insulin. The rate of leptin synthesis by adipocytes may also be affected by the fat depot in which they are located (24), but we have previously shown that there is no effect of fat distribution on plasma leptin levels in the Japanese-American population (15). It is unclear, then, why Japanese Americans with an increased propensity to gain weight also tend to have increased baseline leptin levels. An interesting possibility, though one for which we currently lack evidence, is that the relatively higher levels reflect decreased transport from the blood, including transport into the central nervous system, and are therefore a manifestation of increased leptin resistance.

Several limitations existed in this study. It is not known whether these findings apply to ethnic groups other than Japanese Americans. Our use of CT-measured whole-body adiposity is probably less accurate than underwater weighing. Error due to this measurement, though, is likely to have been random, which would have resulted in an underestimate of the effect of leptin on change in weight and adiposity during follow-up (25). It is possible that unknown confounding factors are responsible for the association between leptin concentration and change in weight or adiposity identified in this study. Such factors could not include age, fasting insulin, or baseline weight or adiposity, since the association was observed after adjustment for these variables.

In conclusion, we found that fat accumulation and weight gain in Japanese Americans are not the result of reduced circulating leptin concentrations. Instead, weight gain and increased adiposity are preceded by relatively higher levels. Although the data presented here are not sufficient to identify the factors that most predispose an individual to subsequent gains in weight and adiposity, our results are consistent with the hypothesis that leptin resistance is a feature of obesity. More work is necessary to elucidate the role of leptin in regulating body weight and adiposity in well-nourished humans.

ACKNOWLEDGMENTS

This work was supported by the ZymoGenetics Corporation. Support also came from National Institutes of Health Grants DK-31170, HL-49293, and HL-47151 (S.D.C.) and from facilities and services provided by the Diabetes and Endocrinology Research Center (DK-17047), the Clinical Nutrition Research Unit (DK-35816), and the General Clinical Research Center (RR-00037) at the University of Washington.

REFERENCES

1. Considine RV, Considine EL, Williams CJ, Nyce MR, Magosin SA, Bauer TL, Rosato EL, Colberg J, Caro JF: Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. *J Clin Invest* 95:2985–2988, 1995
2. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S: Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1:1155–1161, 1996
3. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1995
4. Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV: Leptin: the tale of an obesity gene. *Diabetes* 45:1455–1462, 1996
5. Spiegelman BM, Flier JS: Adipogenesis and obesity: rounding out the big picture. *Cell* 87:377–389, 1996
6. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D: Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 2:589–593, 1996
7. Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M, Caro JF: Response of leptin to short-term fasting and refeeding in humans. *Diabetes* 45:1511–1515, 1996
8. 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
9. Schwartz MW, Brunzell JD: Regulation of body adiposity and the problem of obesity. *Arterioscler Thromb Vasc Biol* 17:233–238, 1997
10. Maffei M, Stoffel M, Barone M, Moon B, Dammerman M, Ravussin E, Bogardus C, Ludwig DS, Flier JS, Talley M, Auerbach S, Friedman JM: Absence of

mutations in the human OB gene in obese/diabetic subjects. *Diabetes* 45:679–682, 1996
11. Ravussin E, Pratley RE, Maffei M, Wang H, Friedman JM, Bennett PH, Bogardus C: Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nat Med* 3:238–240, 1997
12. Fujimoto WY, Leonetti DL, Kinyoun JL, Newell-Morris L, Shuman WP, Stolov WC, Wahl PW: Prevalence of diabetes mellitus and impaired glucose tolerance among second-generation Japanese-American men. *Diabetes* 36:721–729, 1987
13. Boyko EJ, Leonetti DL, Bergstrom RW, Newell-Morris L, Fujimoto WY: Low insulin secretion and high fasting insulin and C-peptide levels predict increased visceral adiposity: 5-year follow-up among initially non-diabetic Japanese-American men. *Diabetes* 45:1010–1015, 1996
14. Shuman WP, Newell-Morris LL, Leonetti DL, Wahl PW, Moceri VM, Moss AA, Fujimoto WY: Abnormal body fat distribution by CT in diabetic men. *Invest Radiol* 21:483–487, 1986
15. Weigle DS, Ganter SL, Kuijper JL, Leonetti DL, Boyko EJ, Fujimoto WY: Effect of regional fat distribution and Prader-Willi syndrome on plasma leptin levels. *J Clin Endocrinol Metab* 82:566–570, 1997
16. Zhongmin M, Gingerich RL, Santiago JV, Klein S, Smith CH, Landt M: Radioimmunoassay of leptin in human plasma. *Clin Chem* 42:942–946, 1996
17. Armitage P, Berry G: *Statistical Methods in Medical Research*. 2nd ed. Oxford, U.K., Blackwell, 1987
18. Kleinbaum DG, Kupper LI, Muller KE: *Applied Regression Analysis and Other Multivariable Methods*. Boston, MA, PWS-Kent, 1988
19. Mooney CZ, Duval RD: *Bootstrapping: A Non-Parametric Approach to Statistical Inference*. Newbury Park, CA, Sage, 1993
20. Saad MF, Damani S, Gingerich RL, Riad-Gabriel MG, Khan A, Boyadjian R, Jinagouda SD, El-Tawil K, Rude RK, Kamdar V: Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab* 82:579–584, 1997
21. Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, Landt M: Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* 45:695–698, 1996
22. Hickey MS, Israel RG, Gardiner SN, Considine RV, McCammon MR, Tyndall GL, Houmard JA, Marks RH, Caro JF: Gender differences in serum leptin levels in humans. *Biochem Mol Med* 59:1–6, 1996
23. 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
24. Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S: Depot- and sex-specific differences in human leptin mRNA expression. *Diabetes* 46:342–347, 1997
25. Rothman KJ: *Modern Epidemiology*. Boston, MA, Little, Brown, 1986, p. 86–89