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# Gender Differences in Serum Leptin Levels in Humans

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**Leptin, the product of the *ob* gene, is an adipose tissue-derived hormone that appears to regulate both satiety and thermogenesis. In the present report, we have reexamined the relationship between circulating leptin concentration and body fat in humans using a more valid measure of adiposity (hydrodensitometry) and have extended these observations to examine the influence of regional body fat distribution and cardiorespiratory fitness. Fasting serum leptin concentration was 6.9 ± 0.3 ng · ml$^{-1}$ in males ($N$ = 333) and 15.2 ± 1.3 ng · ml$^{-1}$ in females ($N$ = 63). Interestingly, total fat mass did not differ between groups (males 20.5 ± 0.5 kg; females 20.4 ± 1.5 kg), suggesting that females have higher leptin levels per unit fat mass. In a multiple regression model, fat mass was the best predictor of serum leptin concentration in males, accounting for 51% of the variance in leptin concentration. In females, percentage body fat was the best predictor of leptin, accounting for 49% of the variance. In both groups, the relationship between leptin and adiposity remained significant after adjusting for age, maximal treadmill time, waist circumference, and fasting insulin concentration. These observations support previous conclusions that circulating leptin is primarily a function of adiposity and demonstrate for the first time that this relationship is independent of fat distribution or cardiorespiratory fitness. The data also suggest that there is a gender dichotomy in the relationship between leptin and body fat mass in humans.** © 1996 Academic Press, Inc.

The recent cloning of the mouse *ob* gene and its human homologue (1) has stimulated considerable interest in the role of adipose tissue as an endocrine organ that may play a significant role in regulating satiety and thermogenesis (2–13). We (4) and others (8,11,12) have reported that human adipose tissue produces a nonmutated form of the *ob* gene. In addition, we (3) have recently reported that serum *ob* protein (leptin) concentration is closely associated with body fat as assessed by either body mass index (BMI) or bioelectrical impedance. However, neither of these procedures are considered to be highly accurate means of assessing adiposity (14,15). Thus, we believe it is imperative to confirm our previous observations using a more valid measure of body fat (hydrodensitometry).

In two recent communications, we reported that neither acute exercise (9) nor 12 weeks of exercise training (10) altered systemic leptin levels in humans. However, these studies were conducted on a relatively small group of subjects, and fat mass was not affected by the exercise protocol in either study. While these observations appear to support a primary role of fat mass per se in contributing to systemic leptin levels, the contribution of cardiorespiratory fitness levels to variations in systemic leptin has not been addressed in a large database. In addition, recent reports suggesting the presence of a gender effect on *ob* expression (11) and circulating leptin levels (16) prompted us to examine the relationship between gender, adiposity, and systemic leptin levels with special reference to the contributions of body fat distribution and cardiorespiratory fitness level.

## METHODS

*Subjects.* We studied 396 subjects who were participants in the Cardiovascular Disease Risk Factor



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**TABLE 1**
**Descriptive Characteristics**

| Variable | Male ($N$ = 333) | Female ($N$ = 63) |
|---|---|---|
| Leptin ($ng \cdot ml^{-1}$) | 6.9 ± 0.3 (1.4–39.9) | 15.2 ± 1.3* (2.8–56.6) |
| Insulin ($pM \cdot liter^{-1}$) | 90.3 ± 2.3 (14.8–270.3) | 83.1 ± 5.5 (18.3–264.9) |
| Fat mass (kg) | 20.5 ± 0.5 (3.8–65.1) | 20.4 ± 1.5 (5.3–58.1) |
| Body fat (%) | 22.3 ± 0.4 (6.1–43.1) | 28.1 ± 1.2* (8.5–47.9) |
| BMI ($kg \cdot m^{-2}$) | 28.2 ± 0.3 (17.6–50.7) | 25.6 ± 0.6* (18.3–44.7) |
| Waist circumference (cm) | 95.6 ± 0.6 (69.0–140.9) | 82.4 ± 1.8* (63.5–123.2) |
| Treadmill time (min) | 18.5 ± 0.3 (2.3–29.5) | 13.3 ± 0.7* (3.0–25.3) |
| Age (year) | 39.1 ± 0.7 (18–75) | 34.4 ± 1.2* (20–62) |
| Height (cm) | 177.3 ± 0.3 (155.6–193.1) | 164.9 ± 0.6* (152.4–177.2) |
| Weight (kg) | 88.7 ± 0.8 (50.9–156.6) | 69.9 ± 1.9* (46.8–123.6) |

*Note.* Data are mean ± SE. Range is in parentheses.
* $P < 0.05$ vs males.

Intervention and Reduction Program at East Carolina University. All subjects were free of cardiovascular disease and were nondiabetic, and none of the subjects were taking any medications.

*Anthropometric tests.* Body density was determined by hydrostatic weighing following expiration to residual volume as determined by oxygen dilution (17). The percentage of body fat, fat mass, and fat-free mass were calculated from body density using the Siri equation (18). Dry body mass was recorded to the nearest 0.1 kg and height to the nearest 0.1 cm. Body mass index (BMI) was calculated as mass/height$^2$ ($kg/m^2$). Waist circumference was obtained as described by Lohman *et al.* (19), as this appears to be the best simple measure of visceral adipose tissue (20). All circumferences were obtained with a spring-tension, stretchless Gulick tape (Lafayette Instruments, Lafayette, IN) to the nearest 1 mm.

*Cardiorespiratory fitness.* Cardiorespiratory fitness was determined from time to exhaustion during a standard (3.3 mph Balke protocol) incremental treadmill test (21).

*Biochemical analysis.* Fasting serum leptin and insulin levels were determined by radioimmunoassay as previously described (3). Serum cholesterol, HDL, LDL, triglycerides, and glucose were determined enzymatically.

*Statistics.* Differences between groups were tested using analysis of variance (ANOVA). Relationships between fasting leptin concentration and selected physiological variables were examined using simple linear regression analysis. In addition, step-wise multiple regression analysis was performed. Because leptin concentration was not normally distributed, all leptin values were log-transformed for analysis. Statistical significance was accepted as $P < 0.05$. All data are reported as mean ± SE.

## RESULTS

Subjects were separated by gender ($N$ = 333 male, 63 female). Descriptive characteristics of the two groups are presented in Table 1. As a group, females had higher leptin levels at any level of fat mass than males (Fig. 1). In females, both the slope (0.635) and the intercept (1.653) were greater than in male subjects (0.422, and −1.837, respectively). Because males had a significantly higher treadmill time vs females, a subgroup of male subjects ($N$ = 63) who were matched for treadmill time to the females was examined. In this group, serum leptin remained significantly lower than in females (9.6 ± 1.0 vs 15.2 ± 1.3 $ng \cdot ml^{-1}$), despite the fact that fat mass was significantly higher in males (26.8 ± 1.6 vs 20.4 ± 1.5 kg, respectively).

Results of simple regression analysis are presented in Table 2. Although a number of simple correlations were observed, in a multiple regression

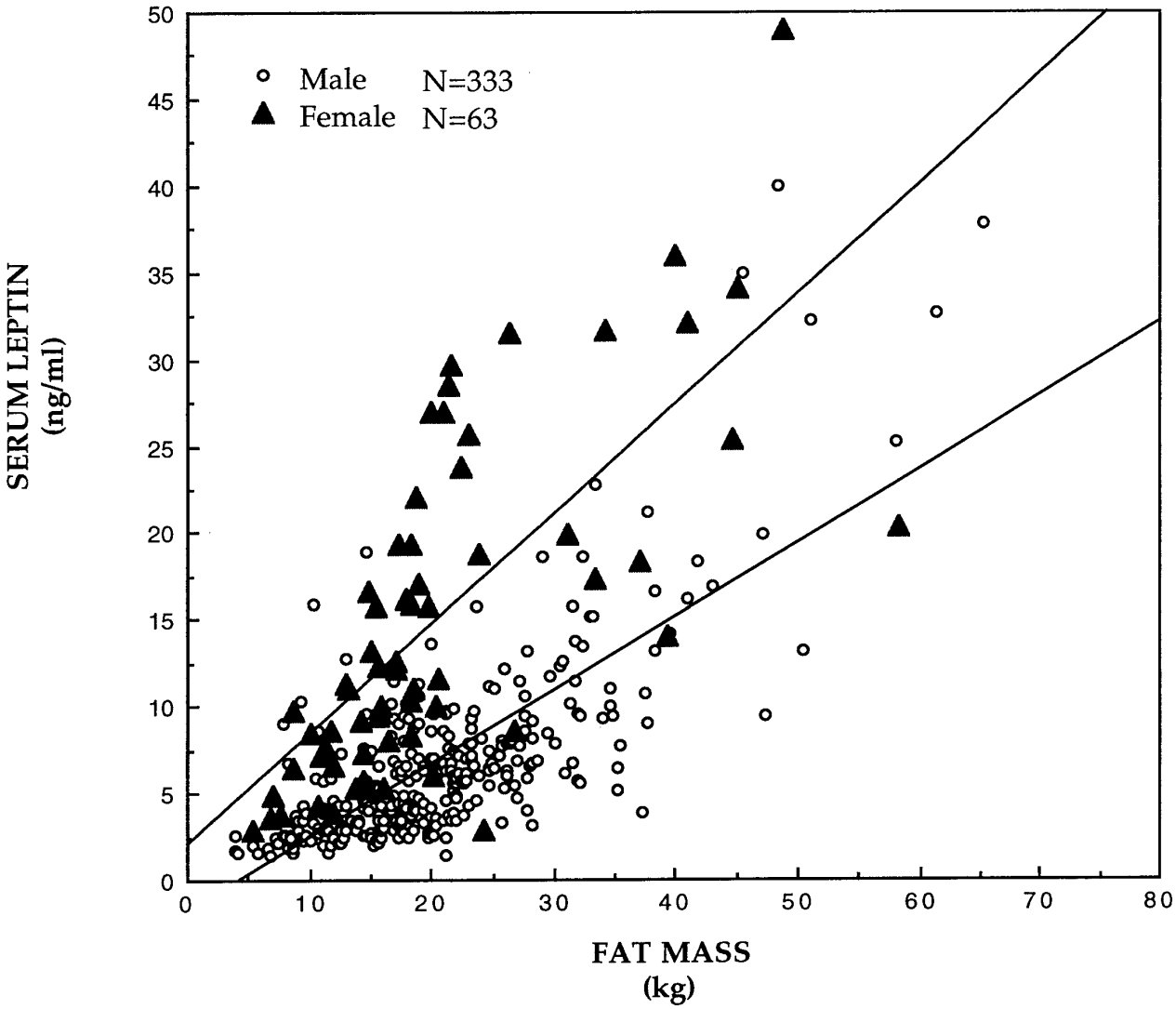


**FIG. 1.** Serum leptin-fat mass relationship. Serum leptin was plotted vs fat mass in male and female subjects. The slope (0.635 vs 0.422) and the intercept (1.653 vs −1.837) of this relationship were greater for females than males.

model, fat mass was the best predictor of leptin levels in male subjects ($r^2$ = 0.515). Fasting insulin entered the equation as step 2 (fat mass + fasting insulin, $r^2$ = 0.529), although it did not contribute substantially to the variance in fasting leptin levels. No other variable entered the equation. The fat mass–leptin relationship in males remained significant after adjusting for age, maximal treadmill time, BMI, and waist circumference (Table 3). In females, percentage fat was the best predictor of leptin levels ($r^2$ = 0.49), although both BMI (% fat + BMI, $r^2$ = 0.53) and fat mass (% fat + BMI + fat mass, $r^2$ = 0.58) contributed to the variance in leptin levels. However, the leptin–percentage fat relationship in females remained significant after adjusting for age, maximal treadmill time, BMI, and waist circumference, but not after adjusting for fat mass (Table 3). In addition, the leptin–insulin relationship in females was no longer significant after adjusting for percentage fat (partial correlation = 0.169).

Finally, serum leptin levels were positively correlated with fasting total cholesterol, LDL cholesterol, triglycerides, and glucose in males subjects, and inversely correlated with HDL cholesterol (Table 2). In females, leptin was positively correlated with total and LDL cholesterol only. None of these relationships remained significant after adjusting for fat mass.

**TABLE 2**
**Regression Analysis**

| Variable | Males | Females |
|---|---|---|
| (log) Leptin vs | | |
| 1. Fat mass | $r = 0.72$* | $r = 0.68$* |
| 2. % Fat | $r = 0.69$* | $r = 0.69$* |
| 3. BMI | $r = 0.66$* | $r = 0.70$* |
| 4. Fasting insulin | $r = 0.45$* | $r = 0.55$* |
| 5. Waist circumference | $r = 0.67$* | $r = 0.65$* |
| 6. Treadmill time | $r = -0.46$* | $r = -0.63$* |
| 7. Age | $r = 0.11$* | $r = 0.23$ |
| 8. Total cholesterol | $r = 0.15$* | $r = 0.28$* |
| 9. HDL cholesterol | $r = -0.22$* | $r = -0.20$ |
| 10. LDL cholesterol | $r = 0.12$* | $r = 0.29$* |
| 11. Triglycerides | $r = 0.12$* | $r = 0.19$* |
| 12. Glucose | $r = 0.12$* | $r = 0.22$ |

* $P < 0.05$ for simple correlation.

## DISCUSSION

The concept of a regulatory mechanism within adipose tissue that controls feeding behavior is not new. In 1953, Kennedy postulated that stored body fat may regulate feeding behavior in rodents (22). This "lipostatic" hypothesis has been revisited a number of times, most notably in the parabiosis experiments of Coleman *et al.* in *ob/ob* and *db/db* mice (23). The recent cloning of the murine and human *ob* genes (1) has renewed interest in the role of adipose tissue in regulating energy balance (2–13,16,24). Several groups have reported that the *ob* gene is expressed in a nonmutated form in human adipose tissue (4,8,11,12). In addition, we recently reported that leptin, the product of the *ob* gene, is elevated in serum from obese humans and is correlated with body fat content as assessed by bioelectrical impedance (3). In the present report, we confirm our previous observation that leptin is related to adiposity, as assessed by hydrodensitometry. This is important in that hydrodensitometry is considered a criterion measurement technique for assessing body fat (14,15). Although it is not without limitations, it is generally accepted to be a better means of assessing adiposity than either BMI or impedance techniques (14,15).

We recently reported in a small group of subjects that females have higher systemic leptin levels than males at any level of body fat (10). The present results also substantiate this observation. The specific biological mechanism responsible for such a gender difference is impossible to determine from the current data. The fact that waist circumference was not an independent contributor to the variance in leptin levels in either group suggests that fat distribution per se cannot account for the observed gender differences. However, there is precedent for gender-dependent differences in adipose tissue metabolism (25), and as such, it is not unreasonable to hypothesize that the profound differences in the hormonal milieu between males and females may result in a differential regulation of *ob* gene expression. In support of this, Lonnqvist *et al.* have reported a 75% increase in *ob* mRNA in adipocytes from females subjects relatives to males (11), and Schwartz *et al.* (16) have reported a similar observation with regard to systemic leptin levels. In addition, Leiter *et al.* have provided evidence that testosterone may play an important role in the phenotypic expression of both the *ob* gene and the *db* gene in mice (26) and that susceptibility to *db*-induced diabetes is strongly influenced by gender (27,28). It must be cautioned that little is known about leptin turnover/clearance in either humans or animals, and we cannot rule out a substantial contribution in terms of leptin catabolism. Lee *et al.* (29) recently reported that the *db* gene, which appears to encode the leptin receptor, has at least six alternatively spliced forms, each of which has a tissue-specific expression pattern. Accordingly, gender-dependent differences in *db* gene expression may contribute to the variation in systemic leptin turnover in humans.

It is of considerable interest to note that neither fat distribution nor cardiorespiratory fitness contributed independently to variations in leptin levels in either gender. Differences in regional adiposity have

**TABLE 3**
**Partial Correlations**

| Variable Adjusted for | Male (log) Leptin vs fat mass | Female (log Leptin vs % fat |
|---|---|---|
| 1. Age | 0.75* | 0.69* |
| 2. Treadmill time | 0.65* | 0.47* |
| 3. BMI | 0.39* | 0.33* |
| 4. Fat Mass | — | 0.23 |
| 5. Waist circumference | 0.42* | 0.42* |
| 6. Fasting insulin | 0.65* | 0.51* |

*Note.* Partial correlations were determined for the leptin:fat mass relationship in males and the leptin:% fat relationship in females after adjusting for other dependent variables.

* Partial correlation is significant after adjusting for variable in question.

been shown to contribute to insulin action and cardiovascular disease risk status in males and females (20,30), and there appear to be profound regional differences in adipose tissue metabolism and hormonal responsiveness even within the same individual (30). However, available evidence does not support a systematic overexpression of *ob* in visceral adipose tissue depots in humans (12). Finally, cardiorespiratory fitness levels, as assessed by maximal treadmill time, were not related to leptin levels after adjusting for adiposity. Further, serum leptin remained ~58% higher in females even when subjects were matched for treadmill time. We have recently reported that neither acute exercise (9) nor exercise training (10) alters systemic leptin levels in humans. The observation that in neither case were there any changes in fat mass suggests that exercise does not have any specific effect on leptin metabolism in the absence of significant differences in adipose tissue mass.

In conclusion, we have substantiated our previous findings that systemic leptin levels in humans are highly correlated with adiposity and additionally report for the first time that this relationship is independent of regional fat distribution and cardiorespiratory fitness. Further, we provide evidence of a gender dichotomy in the leptin–adiposity relationship; females appear to have approximately twofold higher leptin levels at any level of fat mass, and this relationship is also independent of cardiorespiratory fitness levels. The biological mechanisms which contribute to the gender-dependent regulation of leptin metabolism in humans have yet to be elucidated. These areas are clearly potential topics of future research.

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## REFERENCES

1. Zhang Y, Proenca R, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse *obese* gene and its human homologue. *Nature* **372:**425–432, 1994. [*Erratum, Nature* **374:**479, 1995]
2. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269:**546–549, 1995.
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. *New Engl J Med* **334:**292–295, 1996.
4. 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:**2986–2988, 1995.
5. 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.
6. Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, Caro JF. Acute and chronic effect of insulin on leptin production in humans. *Diabetes* **45:**699–701, 1996.
7. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RS, Burley K, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269:**543–546, 1995.
8. Hamilton BS, Paglia D, Kwan AYM, Deitel M. Increased *obese* mRNA expression in omental fat cells from massively obese humans. *Nature Med* **1:**953–956, 1995.
9. Hickey MS, Considine RV, Israel RG, Mahar TL, McCammon MR, Tyndall GL, Houmard JA, Caro JF. Leptin is related to body fat content in male distance runners. *Am. J. Physiol.* In press.
10. Hickey MS, Houmard JA, Considine RV, Tyndall GL, Midyette JB, Gavigan KE, Weidner ML, McCammon MR, Israel RG, Caro JF. Exercise training improves insulin action but does not change serum leptin levels in obese humans. In review.
11. Lonnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the *obese* (*ob*) gene in adipose tissue of human obese subjects. *Nature Med* **1:**950–953, 1995.
12. Masuzaki H, Ogawa Y, Isse N, Satoh N, Okazaki T, Shigemoto M, Mori K, Tamura N, Hosada K, Yoshimasa Y, Jingami H, Kawada T, Nakao K. Human *obese* gene expression. Adipocyte-specific expression and regional differences in adipose tissue. *Diabetes* **44:**855–858, 1995.
13. Pelleymounter MA, Cullen MJ, Baker MB, 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.
14. Hortobagyi T, Israel RG, O'Brien KF. Sensitivity and specificity of the Quetelet index to assess obesity in men and women. *Eur J Clin Nutr* **48:**369–375, 1994.
15. Lohman TG. Advances in body composition assessment. Current Issues in Exercise Science. Monograph No. 3. Champaign, IL: Human Kinetics Books, 1992, p 80.
16. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte Jr, DJ. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Med* **2:**589–593, 1996.
17. Wilmore JA, Vodak P, Parr R, Girandola R, Billing J. Further simplification of a method for determination of residual lung volume. *Med Sci Sports Exercise* **12:**216–218, 1980.
18. Siri WE. The gross composition of the body. Advances in

biological and medical physics. New York: Academic Press, 1956, Vol. IV, p 239.

19. Lohman TG, Roche AF, Martorell A. Eds. Anthropometric Standardization Reference Manual. Champaign, IL: Human Kinetics Books, 1988.
20. Pouliot C-M, Despres J-P, Lemieux S, Moorjani S, Bouchard C, Tremblay A, Nadeau A, Lupien PJ. Waist circumference and abdominal sagittal diameter: Best simple indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol* **73:**460–468, 1994.
21. Pollock ML, Bohannon RL, Cooper KH, Ayres JJ, Ward A, White SR, Linnerud AC. A comparative analysis of four protocols for maximal treadmill stress testing. *Am Heart J* **92:**39–46, 1976.
22. Kennedy GC. The role of depot fat in the hypothalamic control of food intake in the rat. *Proc R Soc London B* **140:**578–592, 1953.
23. Coleman DL. Obese and diabetes: Two mutant genes causing diabetes–obesity syndromes in mice. *Diabetologia* **14:**141–148, 1978.
24. Flier JS. The adipocyte: Storage depot or node on the energy information superhighway? *Cell* **80:**15–18, 1995.
25. Guerre-Millo M, Leturque A, Girard J, Lavau M. Increased insulin sensitivity and responsiveness of glucose metabolism in adipocytes from female vs. male rats. *J Clin Invest* **76:**109–116, 1985.
26. Leiter EH. The genetics of diabetes susceptibility in mice. *FASEB J* **3:**2231–2241, 1989.
27. Leiter EH, Chapman HD, Coleman DL. The influence of genetic background on the expression of mutations at the *diabetes* locus in the mouse. V. Interaction between the *db* gene and hepatic sex steroid sulfotransferases correlates with gender-dependent susceptibility to hyperglycemia. *Endocrinology* **124:**912–922, 1989.
28. Leiter EH, Lee PH, Coleman DL. Susceptibility to *db* gene and streptozotocin-induced diabetes in C57BL mice: Control by gender-associated, MHC-unlinked genes. *Immunogenetics* **26:**6–13, 1987.
29. Lee G-H, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Freidman JM. Abnormal splicing of the leptin receptor in *diabetic* mice. *Nature* **379:**632–635, 1996.
30. Kissebah AH, Krakower GR. Regional adiposity and morbidity. *Physiol Rev* **74**(4):761–811, 1994.