Adipose tissue *ob* mRNA expression in humans: discordance with plasma leptin and relationship with adipose TNFα expression

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Abstract Elevated plasma leptin levels are found in obese humans, suggesting a defect in the function of leptin in regulating body weight and adiposity. In 53 subjects covering a broad range of adiposity, we examined the relationships between plasma leptin, adipose tissue *ob* mRNA levels, and adipose tissue TNF mRNA. There was a highly significant correlation between plasma leptin levels and every index of adiposity. In contrast, the relationship between *ob* mRNA levels and adiposity was weak. Adipose tissue from obese subjects demonstrated higher *ob* mRNA levels than adipose tissue from lean subjects (lean: 0.49 ± 0.05; obese 0.87 ± 0.09 arbitrary units, $P < 0.05$). However, there was no significant correlation between body fat and *ob* mRNA level. In addition, there was no significant relationship between *ob* mRNA levels and plasma leptin levels, which were measured in the same subjects. In addition to the measure of *ob* mRNA levels, adipose TNF mRNA levels were measured in 18 subjects. TNF mRNA levels varied with *ob* mRNA levels ($r = 0.44$, $P = 0.06$). These data show that plasma leptin levels are not directly related to adipose tissue *ob* mRNA levels, suggesting posttranscriptional regulation of leptin expression, either at the level of the adipocyte, or by alteration of plasma leptin degradation or clearance. In addition, the parallel changes in *ob* and TNF mRNA in adipose tissue suggest that these two important factors in the defense against obesity may be regulated similarly.—**Ranganathan, S., M. Maffei, and P. A. Kern.** Adipose tissue *ob* mRNA expression in humans: discordance with plasma leptin and relationship with adipose TNFα expression. *J. Lipid Res.* 1998. **39:** 724–730.

Supplementary key words leptin • obese gene • tumor necrosis factor • obesity • adipose tissue

The defective gene in the *ob/ob* mouse has been described, and the gene product, leptin, is an 167 amino acid protein that is expressed in adipose tissue (1) and placenta (2–4). When *ob/ob* mice are injected with recombinant leptin, weight loss ensues (5–7). The recent description of a receptor for leptin in the hypothalamus (8, 9) supports other studies suggesting that leptin plays an important role in the control of appetite and represents a homeostatic signal intended to limit the expansion of adipose mass.

Several studies have demonstrated that adipose tissue *ob* mRNA and plasma leptin levels are elevated in obese rodents (10–15). In humans, several studies have examined plasma leptin levels in patients with varying levels of adiposity (10, 16–20). Obese subjects generally have higher levels of plasma leptin, suggesting that obese humans are not defective in leptin expression, but rather manifest leptin resistance. Several studies have noted increased *ob* mRNA expression in obese humans (16, 21). Because of the consistent finding of elevated plasma leptin, there is a tendency to assume that plasma leptin levels are directly related to adipose tissue *ob* gene expression.

Another important product of the adipocyte is TNFα, which is elevated in the adipose tissue of obese rodents and humans (22–24), and which may play an important role in obesity-related insulin resistance (25). Although adipose tissue TNF expression is related to obesity, there is much interindividual variation in humans (22), suggesting that other factors control TNF expression.

As described herein, we examined both adipose tissue *ob* and TNF mRNA levels and plasma leptin levels in the same patients. We found a remarkable lack of correlation between adipose tissue expression and plasma leptin, suggesting that other factors are involved in the regulation of plasma leptin levels.

Abbreviations: Abbreviations: TNF, tumor necrosis factor; GAPD, glyceraldehyde phosphate dehydrogenase; BMI, body mass index.

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METHODS

Subjects and patient recruitment

Fifty-three subjects were recruited and 36 underwent an incisional fat biopsy from the lower abdominal wall under local anesthesia. All subjects were weight stable, were taking no medications likely to affect adipocyte metabolism, were non-diabetic, and did not exercise for at least 2 weeks prior to the biopsies. All subjects were placed on a standardized diet consisting of 35% fat, 20% protein, and 45% carbohydrate, calorically adjusted for weight maintenance, for 2 days prior to the study. Body composition was measured using bioelectrical impedance (26). Plasma leptin was measured using an enzyme-linked immunosorbent assay, as described previously (10). Data on some of these subjects have been reported previously (10, 27, 28).

RNA extraction and mRNA analysis

Ob mRNA was measured by Northern blotting, as described previously (10). RNA was extracted using the method of Chomczynski and Sacchi (29) with minor modifications (30). Equal amounts of total RNA were resolved on a 2.2 m formaldehyde–1% agarose gel, transferred to nylon membrane, and blotted with the [^{32}P-labeled cDNA probes for human *ob* (1), and glyceraldehyde phosphate dehydrogenase (GAPD). To further demonstrate that equal quantities of total RNA were loaded, samples of the RNA were resolved by electrophoresis and stained with ethidium bromide for quantitation of the 28S and 18S rRNA bands. To quantitate differences between Northern blots, autoradiographic images were quantitated by densitometry on a laser image analyzer.

TNF mRNA was measured using competitive RT-PCR, as described previously (22). In brief, 0.4 μg of total RNA from adipose tissue was added to increasing quantities of a competing RNA construct (31), which contained primer sites for human TNF, but which contained a 49 nt internal deletion. After the reverse transcriptase reaction, PCR was carried out for 35 cycles at 60°C, and the resulting PCR products were resolved on a 2% agarose gel and the ethidium bromide-stained gel was quantitated. The ratio of TNF product/cRNA standard was plotted against the number of copies of cRNA added, to yield the equivalence point between cRNA and TNF mRNA. Data are expressed as the "number of copies"/μg of total RNA, were "number of copies" refers to the number of copies of the cRNA added.

Statistics

Data are expressed as the mean ± SEM. Data were analyzed non-parametrically, using the Mann-Whitney test for non-paired data. Linear regressions were performed using the Pearson product correlation coefficient.

RESULTS

As described by us and others, plasma leptin correlated significantly with indicators of adiposity. Such was the case with the subjects in this study. There was a significant relationship between plasma leptin and body mass index (BMI) ($r = 0.711$, $P < 0.0001$), and between plasma leptin and total body fat ($r = 0.761$, $P < 0.0001$).

In previous studies, a significant relationship was observed between *ob* mRNA levels and adiposity (16, 21). The *ob* mRNA levels from our subjects were analyzed with regard to a number of factors. When patients were divided into lean and obese (lean was defined as <20% fat in men, and <30% fat in women), *ob* mRNA levels were significantly higher in obese subjects (**Fig. 1**). However, *ob* mRNA levels did not increase consistently with increased adiposity. As shown in **Fig. 2**, a regression of *ob* mRNA levels with percent body fat showed no significant relationship. Similarly, there was no significant relationship between other indices of adiposity and *ob* mRNA levels. The correlation coefficient between % body fat and *ob* mRNA was 0.18, and between total fat mass and *ob* mRNA level, the correlation coefficient was 0.05. Of the 36 subjects who underwent fat biopsies, 27 were women. However, the analysis of women and men separately did not improve the correlation between adiposity and *ob* mRNA levels nor did the relationship improve by excluding the very obese (BMI > 40) subjects. Both plasma leptin and *ob* mRNA were measured in subjects, and the relationship between these measurements is shown in **Fig. 3**. There was no relationship between the mRNA expression of *ob*, and the plasma level of the leptin protein. In **Fig. 4**, representative Northern blots are shown to clearly illustrate these points. As shown by the blots and the corresponding plasma leptin levels below each lane, patients with the highest plasma leptin levels did not necessarily have the highest *ob* expression. Even within the same subjects, the changes in *ob* mRNA and leptin were poor. As described by us previously (10), weight loss resulted in a consistent decrease in plasma leptin, and a variable decrease in *ob* mRNA levels. In 8 subjects who had measurements of leptin and *ob* mRNA both before and after weight loss, the correlation between the percent changes in leptin and *ob* mRNA was not significant ($r = 0.08$).

Obese rodents and humans express high levels of

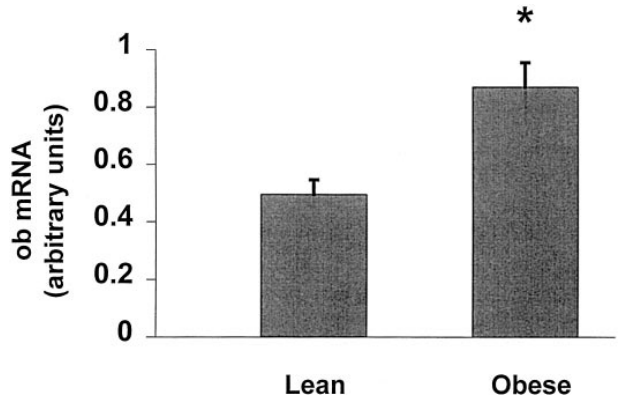

Fig. 1. *Ob* mRNA levels in lean and obese subjects. Subjects were divided into lean and obese based on % of body fat (see methods). *$P < 0.01$.

TNF in adipose tissue, which may play a role in the development of insulin resistance. In the adipose tissues of 18 subjects, we measured TNF mRNA using competitive RT-PCR. As shown in **Fig. 5**, adipose tissue TNF expression varied with adipose tissue *ob* mRNA levels ($r = 0.44$, n = 18, $P = 0.06$). There was no relationship between plasma leptin levels and adipose TNF mRNA expression ($r = 0.01$, P = NS).

DISCUSSION

Since the original description of the *ob* gene and the corresponding plasma protein (1), numerous studies have analyzed *ob* regulation and plasma leptin levels. Plasma leptin levels are strongly associated with total adipose tissue mass. Not only is plasma leptin increased in obese subjects (10, 16, 18–20), and decreased in reduced-obese subjects (10, 32), but leptin is decreased adipose tissue mass induced by exercise (33, 34) and anorexia nervosa (35). Fewer studies have examined the relationship between adipose tissue *ob* mRNA levels and adiposity. Using a PCR method, Considine et al. (16) measured *ob* mRNA levels in 54 subjects and found a highly significant correlation with BMI and % body fat. Several studies also found an increase in *ob* mRNA levels in obese patients (21, 32, 36). However, no previous study has directly compared *ob* mRNA levels with plasma leptin levels in the same subjects.

In this study, we found that obese subjects as a group demonstrated higher levels of *ob* mRNA than lean subjects; however, there was no significant correlation between *ob* mRNA levels and adiposity using either BMI, percent body fat, or total body fat. In addition, we found no significant relationship between adipose tissue *ob* mRNA level and plasma leptin level. However, as with numerous other studies, we found the same significant relationship between adiposity and plasma leptin levels.

Previous studies on *ob* mRNA differed from this one in several ways. Isolated adipocytes were used after a collagenase digestion in some studies (16), whereas we used whole adipose tissue. As the *ob* mRNA is only present at very low levels in preadipocytes (37), either method should yield similar results unless there was selective loss of an adipocyte population during the collagenase digestion. Some studies used PCR for the RNA measurement (16, 32), although without an internal standard to control for the reverse transcriptase reaction and variations in the thermocycling.

In this and most other studies, subcutaneous abdominal adipose tissue was sampled. It is possible that this adipose depot is not representative of plasma leptin

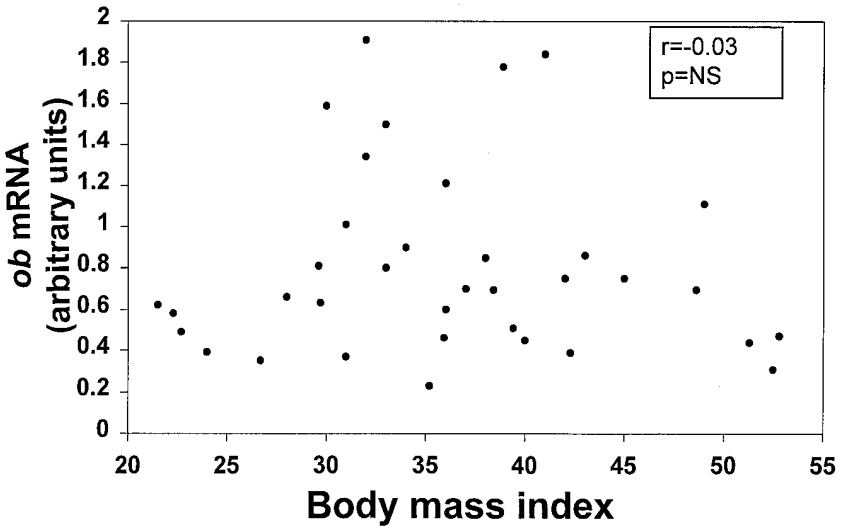

Fig. 2. Relationship between *ob* mRNA levels and BMI. *Ob* mRNA levels are shown in relation to each subject's BMI.

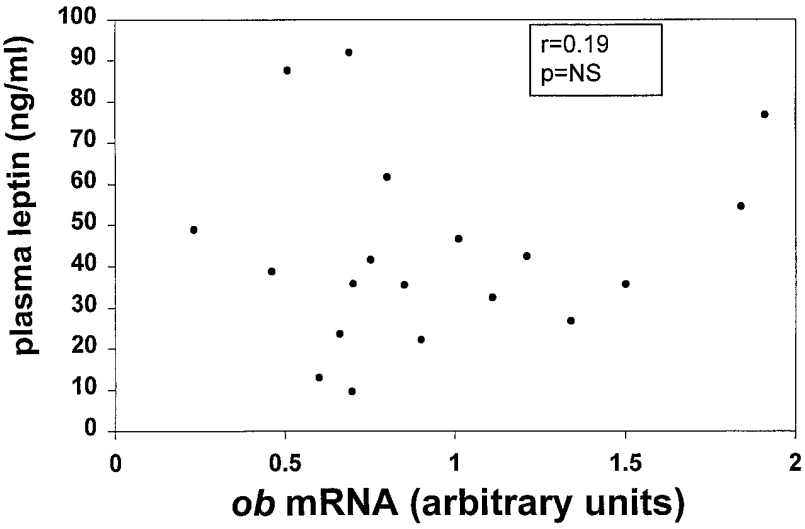

Fig. 3. Relationship between *ob* mRNA level and plasma leptin. *Ob* mRNA/GAPD ratio is shown in relation to the plasma leptin level for each subject.

and hence the RNA and protein data appear unrelated. Based on other studies, however, this is unlikely. Depending on the study, 74–93% of total body adipose tissue is found in subcutaneous adipose tissue using MRI or CT with careful quantitation (38–40). Although the proportion of visceral adipose tissue is a little higher in men, all studies show that the large majority of total body adipose mass is located subcutaneously. Therefore, because subcutaneous adipose tissue makes up the bulk of adipose mass, it is highly unlikely that some other adipose tissue depot could contribute significantly to leptin expression.

The lack of correlation between adipose tissue *ob* mRNA and plasma leptin suggests that the regulation of *ob* gene expression may be complex, and plasma leptin levels may not be dependent solely on adipose tissue *ob* mRNA expression. The high levels of plasma leptin in obese subjects could be due to posttranscriptional mechanisms, such as leptin translation or secretion from the adipocyte. A recent study provided evidence for such posttranscriptional mechanisms. Kirchgessner et al. (41) found that plasma leptin was decreased, with no change in leptin mRNA levels, in TNF knockout mice. In addition, 3T3-L1 cells demonstrated a discordance between leptin secretion and mRNA levels (41). An additional potential site for leptin regulation is the degradation of the plasma protein. Previous studies have suggested that leptin has a short plasma half-life (42, 43) of less than 24 min, and is bound to other proteins, thus raising the possibility for regulation of leptin

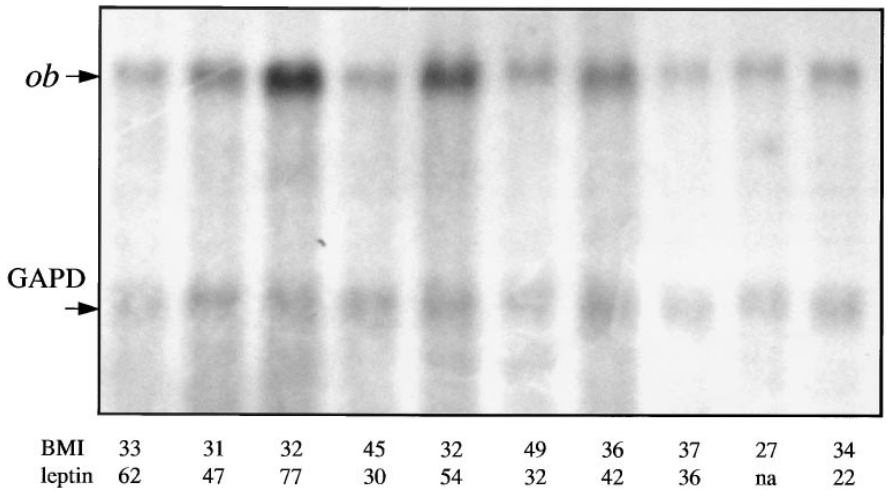

Fig. 4. Representative Northern blots. Two representative Northern blots are shown, with each subject's BMI and plasma leptin shown below the lanes.

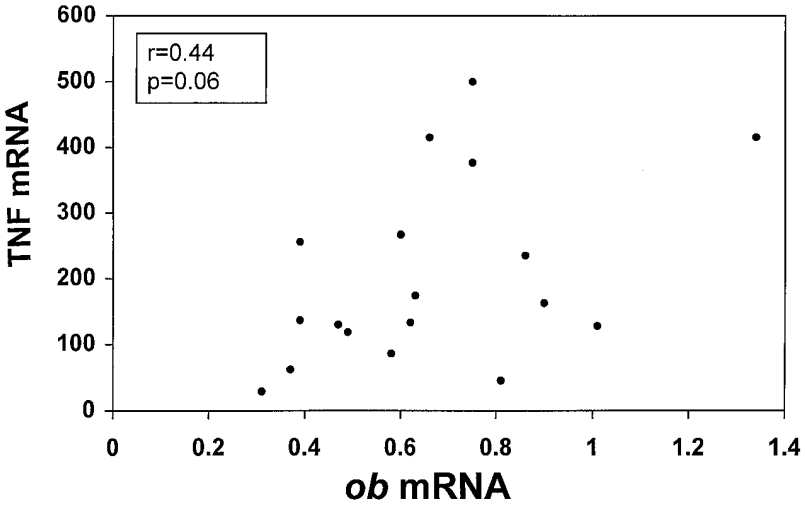

Fig. 5. Relationship between *ob* mRNA and TNF mRNA levels in 18 subjects covering a wide range of adiposity.

degradation or availability (44). However, no changes were found in leptin half-life in obese subjects (42). In addition, when adipose tissue leptin production was measured by comparing abdominal venous leptin levels with peripheral leptin levels, a direct correlation between abdominal adipose leptin production and obesity was found (42). Another possible explanation for the apparent differences between studies is patient heterogeneity. Although several studies have observed a decrease in *ob* mRNA after weight loss (10, 16), this finding was not consistent, suggesting that some subjects decrease plasma leptin through posttranscriptional mechanisms.

We also observed a correlation between adipose tissue *ob* mRNA and adipose tissue TNF expression. Adipose tissue TNF expression increases with increasing adiposity in humans (22, 24) and has been implicated in the insulin resistance of obesity (23, 25). Other studies have previously noted a relationship between TNF and leptin expression. When TNF was injected into mice and hamsters, the anorexia was accompanied by an increase in plasma leptin and adipose leptin mRNA levels within 8 h (45, 46). In a recent study, leptin secretion by 3T3-L1 cells was stimulated by the addition of TNF (41). In addition, TNF knockout mice had lower levels of plasma leptin than controls (41). Although the relationship between TNF and leptin expression in humans was of borderline statistical significance, these data tend to corroborate the findings noted above in rodents. One possible explanation for the parallel increase in TNF and *ob* mRNA in obese humans relates to the overall defense of the animal against the deleterious effects of obesity. Thus, it is possible that both adipose TNF expression and *ob* mRNA expression are different parts of the defense against obesity: one working to control appetite, and the other (TNF) making the organism more insulin resistant.

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REFERENCES

1. Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature.* **372:** 425–432.
2. Senaris, R., T. Garciacaballero, X. Casabiell, R. Gallego, R. Castro, R. V. Considine, C. Dieguez, and F. F. Casanueva. 1997. Synthesis of leptin in human placenta. *Endocrinology.* **138:** 4501–4504.
3. Masuzaki, H., Y. Ogawa, N. Sagawa, K. Hosoda, T. Matsumoto, H. Mise, H. Nishimura, Y. Yoshimasa, I. Tanaka, T. Mori, and K. Nakao. 1997. Nonadipose tissue production of leptin. Leptin as a novel placenta-derived hormone in humans. *Nature Med.* **3:** 1029–1033.
4. Hoggard, N., L. Hunter, J. S. Duncan, L. M. Williams, P. Trayhurn, and J. G. Mercer. 1997. Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. *Proc. Natl. Acad. Sci. USA.* **94:** 11073–11078.
5. Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley, and J. M. Friedman. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science.* **269:** 543–546.
6. Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos, and P. Burn. 1995. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science.* **269:** 546–549.
7. Pelleymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins. 1995. Effects of the

obese gene product on body weight regulation in *ob/ob* mice. *Science.* **269:** 540–543.
8. Tartaglia, L. A., M. Dembski, X. Weng, N. H. Deng, J. Culpepper, R. Devos, G. J. Richards, L. A. Campfield, F. T. Clark, J. Deeds, C. Muir, S. Sanker, A. Moriarity, K. J. Moore, J. S. Smutko, G. G. Mays, E. A. Woolf, C. A. Monroe, and R. I. Tepper. 1995. Identification and expression cloning of a leptin receptor, Ob-R. *Cell.* **83:** 1263–1271.
9. Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G. Darvishzadeh, J. I. Lee, and J. M. Friedman. 1996. Abnormal splicing of the leptin receptor in diabetic mice. *Nature.* **379:** 632–635.
10. Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, P. A. Kern, and J. M. Friedman. 1995. Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nature Med.* **1:** 1155–1161.
11. Hardie, L. J., D. V. Rayner, S. Holmes, and P. Trayhurn. 1996. Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (*fa/fa*) rats as measured by ELISA. *Biochem. Biophys. Res. Commun.* **223:** 660–665.
12. Igel, M., H. Kainulainen, A. Brauers, W. Becker, L. Herberg, and H. G. Joost. 1996. Long-term and rapid regulation of *Ob* mRNA levels in adipose tissue from normal (Sprague Dawley rats) and obese (*db/db* mice, *fa/fa* rats) rodents. *Diabetologia.* **39:** 758–765.
13. Sivitz, W. I., H. L. Bailey, and P. Donohoue. 1996. Rat adipose *ob* mRNA levels in states of altered circulating glucose and insulin. *Biochem. Biophys. Res. Commun.* **220:** 520–525.
14. Frederich, R. C., B. Loellmann, A. Hamann, A. Napolitano-Rosen, B. B. Kahn, B. B. Lowell, and J. S. Flier. 1995. Expression of *ob* mRNA and its encoded protein in rodents: impact of nutrition and obesity. *J. Clin. Invest.* **96:** 1658–1663.
15. Ogawa, Y., H. Masuzaki, N. Isse, T. Okazaki, K. Mori, M. Shigemoto, N. Satoh, N. Tamura, K. Hosoda, Y. Yoshimasa, H. Jingami, T. Kawada, and K. Nakao. 1995. Molecular cloning of rat obese cDNA and augmented gene expression in genetically obese Zucker fatty (*fa/fa*) rats. *J. Clin. Invest.* **96:** 1647–1652.
16. Considine, R. V., M. K. Sinha, M. L. Heiman, A. Kriauciunas, T. W. Stephens, M. R. Nyce, J. P. Ohannesian, C. C. Marco, L. J. Mckee, T. L. Bauer, and J. F. Caro, 1996. Serum immunoreactive leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med.* **334:** 292–295.
17. Dagogojack, S., C. Fanelli, D. Paramore, J. Brothers, and M. Landt. 1996. Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes.* **45:** 695–698.
18. Haffner, S. M., R. L. Gingerich, H. Miettinen, and M. P. Stern. 1996. Leptin concentrations in relation to overall adiposity and regional body fat distribution in Mexican Americans. *Int. J. Obes.* **20:** 904–908.
19. Ashley, C. T., Jr., K. D. Wilkinson, D. Reines, and S. T. Warren. 1996. *FMR1* protein: conserved RNP family domains and selective RNA binding. *Science.* **262:** 563–566.
20. Ostlund, R. E., J. W. Yang, S. Klein, and R. Gingerich. 1996. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J. Clin. Endocrinol. Metab.* **81:** 3909–3913.
21. Vidal, H., D. Auboeuf, P. De Vos, B. Staels, J. P. Riou, J. Auwerx, and M. Laville. 1996. 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.
22. Kern, P. A., M. Saghizadeh, J. M. Ong, R. J. Bosch, R. Deem, and R. B. Simsolo. 1995. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J. Clin. Invest.* **95:** 2111–2119.
23. Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. Adipose expression of tumor necrosis factor-a: direct role in obesity-linked insulin resistance. *Science.* **259:** 87–91.
24. Hotamisligil, G. S., P. Arner, J. F. Caro, R. L. Atkinson, and B. M. Spiegelman. 1995. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J. Clin. Invest.* **95:** 2409–2415.
25. Hotamisligil, G. S. and B. M. Spiegelman. 1994. Tumor necrosis factor a: a key component of the obesity–diabetes link. [Review]. *Diabetes.* **43:** 1271–1278.
26. Segal, K. R., B. Gutin, E. Presta, J. Wang, and T. B. Van Itallie. 1985. Estimation of human body composition by electrical impedance methods: a comparative study. *J. Appl. Physiol.* **58:** 1565–1571.
27. Ong, J. M., and P. A. Kern. 1989. Effect of feeding and obesity on lipoprotein lipase activity, immunoreactive protein, and messenger RNA levels in human adipose tissue. *J. Clin. Invest.* **84:** 305–311.
28. Simsolo, R. B., J. M. Ong, and P. A. Kern. 1993. The regulation of adipose tissue and muscle lipoprotein lipase in runners by detraining. *J. Clin. Invest.* **92:** 2124–2130.
29. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal. Biochem.* **162:** 156–159.
30. Saffari, B., J. M. Ong, and P. A. Kern. 1992. Regulation of adipose tissue lipoprotein lipase gene expression by thyroid hormone in rats. *J. Lipid Res.* **33:** 241–249.
31. Andus, T., S. R. Targan, R. Deem, and H. Toyoda. 1993. Measurement of tumor necrosis factor alpha mRNA in small numbers of cells by quantitative polymerase chain reaction. *Reg. Immunol.* **5:** 11–17.
32. Considine, R. V., E. L. Considine, C. J. Williams, M. R. Nyce, S. A. Magosin, T. L. Bauer, E. L. Rosato, J. Colberg, and J. F. Caro. 1995. Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity [see comments]. *J. Clin. Invest.* **95:** 2986–2988.
33. Kohrt, W. M., M. Landt, and S. J. Birge. 1996. Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. *J. Clin. Endocrinol. Metab.* **81:** 3980–3985.
34. Hickey, M. S., R. V. Considine, R. G. Israel, T. L. Mahar, M. R. McCammon, G. L. Tyndall, J. A. Houmard, and J. F. Caro. 1996. Leptin is related to body fat content in male distance runners. *Am. J. Physiol.* **34:** E938–E940.
35. Grinspoon, S., T. Gulick, H. Askari, M. Landt, K. Lee, E. Anderson, Z. M. Ma, L. Vignati, R. Bowsher, D. Herzog, and A. Klibanski. 1996. Serum leptin levels in women with anorexia nervosa. *J. Clin. Endocrinol. Metab.* **81:** 3861–3863.
36. Hamilton, B. S., D. Paglia, A. Y. M. Kwan, and M. Deitel. 1995. Increased obese mRNA expression in omental fat cells from massively obese humans. *Nature Med.* **1:** 953–956.
37. Wabitsch, M., P. B. Jensen, W. F. Blum, C. T. Christoffer-

sen, P. Englaro, E. Heinze, W. Rascher, W. Teller, H. Tornqvist, and H. Hauner. 1996. Insulin and cortisol promote leptin production in cultured human fat cells. *Diabetes.* **45:** 1435–1438.
38. Abate, N., A. Garg, R. M. Peshock, J. Stray-Gundersen, B. Adams-Huet, and S. M. Grundy. 1996. Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM. *Diabetes.* **45:** 1684–1693.
39. Ross, R., K. D. Shaw, Y. Martel, J. De Guise, and L. Avruch. 1993. Adipose tissue distribution measured by magnetic resonance imaging in obese women. *Am. J. Clin. Nutr.* **57:** 470–475.
40. Sohlström, A., L-O. Wahlund, and E. Forsum. 1993. Adipose tissue distribution as assessed by magnetic resonance imaging and total body fat by magnetic resonance imaging, underwater weighing, and body–water dilution in healthy women. *Am. J. Clin. Nutr.* **58:** 830–838.
41. Kirchgessner, T. G., K. T. Uysal, S. M. Wiesbrock, M. Marino, and G. S. Hotamisligil. 1997. Tumor necrosis factor-alpha contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. *J. Clin. Invest.* In press.
42. Klein, S., S. W. Coppack, V. Mohamedali, and M. Landt. 1996. Adipose tissue leptin production and plasma leptin kinetics in humans. *Diabetes.* **45:** 984–987.
43. Cumin, F., H. P. Baum, and N. Levens. 1996. Leptin is cleared from the circulation primarily by the kidney. *Int. J. Obes.* **20:** 1120–1126.
44. Sinha, M. K., I. Opentanova, J. P. Ohannesian, J. W. Kolaczynski, M. L. Heiman, J. Hale, G. W. Becker, R. R. Bowsher, T. W. Stephens, and J. F. Caro. 1986. Evidence of free and bound leptin in human circulation—studies in lean and obese subjects during short-term fasting. *J. Clin. Invest.* **98:** 1277–1282.
45. Grunfeld, C., C. Zhao, J. Fuller, A. Pollock, A. Moser, J. Friedman, and K. R. Feingold. 1996. Endotoxin and cytokines induce expression of leptin, the *ob* gene product, in hamsters. A role for leptin in the anorexia of infection. *J. Clin. Invest.* **97:** 2152–2157.
46. Sarraf, P., R. C. Frederich, E. M. Turner, G. Ma, N. T. Jaskowiak, D. J. Rivet, J. S. Flier, B. B. Lowell, D. L. Fraker, and H. R. Alexander. 1997. Multiple cytokines and acute inflammation raise mouse leptin levels—potential role of inflammatory anorexia. *J. Exp. Med.* **185:** 171–175.