Determinants of leptin gene expression in fat depots of lean mice

YIYING ZHANG,1 KAI-YING GUO,1 PATRICIA A. DIAZ,1 MOONSEONG HEO,2 AND RUDOLPH L. LEIBEL1
1Division of Molecular Genetics, Department of Pediatrics, and the Naomi Berrie Diabetes Center, Columbia University College of Physicians and Surgeons, New York 10032; and 2New York Obesity Research Center, St. Luke’s-Roosevelt Hospital, Columbia University College of Physicians and Surgeons, New York, New York 10025

Received 9 July 2001; accepted in final form 24 September 2001

Determinants of leptin gene expression in fat depots of lean mice. Am J Physiol Regulatory Integrative Comp Physiol 282: R226–R234, 2002; 10.1152/ajpregu.00392.2001.—The relationship of leptin gene expression to adipocyte volume was investigated in lean 10-wk-old male C57BL/6J mice. mRNA levels for leptin, insulin receptor, glucocorticoid receptor, and tumor necrosis factor (TNF)-α in inguinal, epididymal, and retroperitoneal adipose tissues were quantified and related to adipocyte volume. Leptin mRNA levels were highly correlated with adipocyte volume within each fat depot. Multiple regression analysis of pooled data from the three depots showed that leptin mRNA levels were strongly correlated with adipocyte volumes (β = 0.84, P < 0.001) and, to a smaller degree, with glucocorticoid receptor mRNA levels (β = 0.36, P < 0.001). Depot of origin had no effect (P > 0.9). Rates of leptin secretion in vitro were strongly correlated with leptin mRNA levels (r = 0.89, P < 0.001). mRNA levels for TNF-α, insulin receptor, and glucocorticoid receptor showed no significant correlation with adipocyte volume. These results demonstrate that depot-specific differences in leptin gene expression are mainly related to the volumes of the constituent adipocytes. The strong correlation between leptin gene expression and adipocyte volume supports leptin’s physiological role as a humoral signal of fat mass.

glucocorticoid receptor; insulin receptor; tumor necrosis factor-α

A CRITICAL QUESTION in the physiology of body weight regulation is how the size of somatic energy stores (fat mass) is signaled to the brain and other organs. Leptin is a compelling candidate for such a signal, in part, because its plasma concentration is highly correlated with adiposity in both humans and rodents (7, 15, 33, 44, 49). However, because of evident depot-specific differences in leptin gene expression (23, 30, 34, 37, 41, 45, 59), it is not clear how leptin production from each fat depot is coordinated to provide an accurate signal with regard to the total fat mass.

Depot-specific differences in leptin gene expression have been observed in both human and rodent adipocytes (23, 30, 34, 37, 41, 45, 59). Leptin mRNA levels are higher in rat gonadal and retroperitoneal (intra-abdominal depots) than in inguinal (subcutaneous) adipose tissues (30, 41, 59). In humans, leptin mRNA levels and secretion rates are three- to fivefold higher in subcutaneous than omental adipose tissue (23, 34, 37, 45, 52). Because omental adipocytes are smaller than subcutaneous adipocytes in humans, such depot-specific differences in leptin gene expression may be related to differences in adipocyte size (23, 52). Consistent with this idea, leptin mRNA levels and rates of leptin secretion in both subcutaneous and omental adipose tissues are positively correlated with body mass index, which is usually correlated with adipocyte volume (25, 52). Contrary to these findings, leptin mRNA levels and secretion rates are higher in subcutaneous adipocytes than in omental adipocytes from obese human subjects, even though average adipocyte volumes are similar between the two depots (45), suggesting that adipocyte size may not be the main determinant of leptin gene expression level in large adipocytes from severely obese patients. In addition, short-term fasting dramatically decreases plasma leptin and adipose tissue leptin mRNA before having significant effects on total adiposity or adipocyte size in both humans and rodents (43, 47, 59), suggesting that acute energy balance also plays an important role in determining leptin gene expression. Such effects may be mediated, in part, by circulating concentrations of insulin, glucocorticoids, and catecholamines.

In rodents, insulin administration increases leptin gene expression during fasting and prevents the decrease of leptin mRNA and protein levels induced by caloric restriction (47, 59). Insulin also increases leptin mRNA levels in 3T3-L1 and 3T3-F442A adipocytes in vitro (29, 32). However, insulin appears to have no acute effect on leptin mRNA levels in adipocytes isolated from fed rats in vitro (4, 47). Prolonged infusion of

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
exogenous insulin in the context of a euglycemic clamp increases plasma leptin concentrations in humans (2, 25). Acute effects of insulin infusion at physiological concentrations in the presence of a euglycemic clamp on leptin production in human subjects appear to be more variable, ranging from no effect to increased plasma leptin concentration (2, 25, 46). It has been suggested that the effects of insulin on leptin gene expression are secondary to its effects on glucose and lipid metabolism (38, 40, 46, 48). Insulin also increases leptin secretion in rat and human adipose tissues in vitro in the absence of significant effects on leptin mRNA levels, suggesting that insulin may have effects on the release of a stored pool of leptin (4, 45).

Glucocorticoids increase leptin mRNA expression and secretion in human and rodent adipocytes both in vivo and in vitro (4, 17, 45, 50, 54). Glucocorticoid receptor mRNA levels are higher in omental (in humans) or epididymal (in rats) than subcutaneous adipocytes (36, 42). Consistent with these results, human omental, relative to subcutaneous, adipose tissues appear to be more responsive to dexamethasone’s effects on leptin gene expression in vitro (45, 54). Together, these results suggest that adipocyte sensitivity to glucocorticoids may play a role in depot-specific differences in leptin gene expression.

Increased tumor necrosis factor (TNF)-α gene expression in adipocytes of obese and aging humans and rodents has been linked to insulin resistance (21, 22). Correlations of adipocyte TNF-α mRNA and activity levels with adiposity and adipocyte volumes have been observed in rats (39), suggesting that the level of gene expression for both TNF-α and leptin may be related to adipocyte volume in a similar way.

In the experiments reported here, we quantified mRNA levels of leptin, insulin receptor, glucocorticoid receptor, and TNF-α and related these to cell volume and rates of leptin secretion in adipocytes from three major fat depots of 10-wk-old C57BL/6J male mice. The relationship of mRNA levels of insulin receptor, glucocorticoid receptor, and TNF-α to leptin mRNA levels was also analyzed. Lean young adult male mice were used to minimize possible confounding effects of obesity, aging, or cyclical changes in gonadal steroids. We conclude that leptin mRNA levels and rates of leptin secretion are highly correlated with adipocyte volume and that adipocyte volume is the major factor accounting for differences in the levels of leptin gene expression among fat depots in lean young mice.

### MATERIALS AND METHODS

**Animals and adipose tissue sample collection.** Male C57BL/6J mice were purchased from Jackson Laboratory at 8 wk of age and were maintained in a barrier facility (12:12-h light-dark cycle) for another 2 wk with ad libitum access to regular rodent chow (Purina rodent chow 5035). Animals were deprived of food for 5 h before death and were killed by CO2 asphyxia at the 8th hour into the light cycle. Adipose tissues from inguinal, epididymal, and retroperitoneal pads were dissected and immediately frozen in liquid nitrogen for RNA extraction. Adipose tissue from the contralateral pad was dissected and fixed in Bouin’s fixative for measurement of adipocyte size in paraffin sections. In another set of animals, these contralateral inguinal or epididymal fat pads were separately pooled and used to measure the rate of leptin secretion and leptin mRNA levels.

**Determination of mRNA levels for leptin, insulin receptor, glucocorticoid receptor, and TNF-α.** Total RNA was extracted from adipose tissue using TriZol reagent ( Gibco, Bethesda, MD). About 1–5 μg of total RNA was reverse-transcribed into cDNA using a random hexamer and M-MLV reverse transcriptase. cDNA was quantitatively amplified by PCR using specific primers for leptin (21 cycles), insulin receptor (22 cycles), glucocorticoid receptor (22 cycles), or TNF-α (28 cycles) in combination with primers for β-actin (17 cycles) (as an internal control) in the first PCR amplification as previously described (58). Amplification of each cDNA was performed on the linear portion of the amplification curve. Primer sequences for all genes tested are listed in Table 1.

**[32P]dCTP-labeled PCR products were visualized and quantified using a Phospholmager (Molecular Dynamics).** mRNA levels for leptin, insulin receptor, glucocorticoid receptor, and TNF-α were expressed as a ratio to β-actin mRNA to normalize for initial RNA input.

**Determination of adipocyte volume.** The average diameter of adipocytes in each tissue sample was determined using a photomicrographic method described by Di Girolamo et al. (10) and validated by Ashwell et al. (1). Briefly, paraffin-embedded adipose tissue was cut into 7-μm sections and processed and stained with hematoxylin and eosin. Fields of representatively sized cells were photographed at ×100 magnification. Care was taken to choose an area with minimal stromal-vascular cells. The total number of cells in a 0.5 × 0.5-mm area (calibrated with micrometer) was tallied in each of three discontinuous sections for every single tissue sample. Cells were counted only if more than one-half of their area fell within the defined perimeter. The mean adipocyte diameter for each sample was calculated on the basis of the average number of cells in the defined area using the following formula: diameter = 1.1 × 2 × [area/(cell number × π)]^1/2. The correction factor of 1.1 was included in the calculation of the average diameter of adipocytes as suggested by Ashwell et al. (1), who compared the result obtained by the

---

**Table 1. Primer sequences for all genes tested**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>TGACACAGAAAAACCCCTCATCA</td>
<td>AGGCCAGAAATGAGATGCAA</td>
</tr>
<tr>
<td>Insulin receptor</td>
<td>GAGACAGATGTGAGACAGGC</td>
<td>CAGGGTCGTCGCGATGTCC</td>
</tr>
<tr>
<td>Glucocorticoid receptor</td>
<td>GACAGCTCTGTTCAGACATGC</td>
<td>GGGTATTGTGCTACTACAGG</td>
</tr>
<tr>
<td>TNF-α</td>
<td>CCACCAAGCTTCTTGCTCGTA</td>
<td>ASCTGCTGTCGCACTCTSTG</td>
</tr>
<tr>
<td>β-Actin</td>
<td>AGGCGGCAAGCAGGAGGAG</td>
<td>GGGTGTGAGAGGCTTCAAAC</td>
</tr>
</tbody>
</table>

TNF-α, tumor necrosis factor-α.
above photomicrographic method to the result obtained by measuring the diameters of adipocytes released from a tissue matrix by collagenase digestion. The average adipocyte volume, expressed as micrograms lipid per cell, was then calculated using the calculated average diameter and lipid density of 0.915 g/ml.

Measurement of the rate of leptin secretion. The rate of leptin secretion by adipose tissue was measured as described by Van Harmelen et al. (52). Briefly, fresh adipose tissue from respective depots of 10 mice was cut into 10- to 15-mg fragments and incubated in Krebs-Ringer bicarbonate buffer (gassed with 5% CO2-95% O2 for 45 min before the incubation) containing 1 g/l glucose and 40 g/l fatty acid-free BSA. Inguinal adipose tissue (150–200 mg) or epididymal adipose tissue (200–300 mg) was incubated in 3 ml of buffer for 2 h at 37°C with constant shaking. After the incubation, 2.5 ml of the incubation medium was lyophilized and resuspended in 0.25 ml of water. Two 0.1-ml aliquots of each sample were assayed in duplicate for leptin using a radioimmunoassay for murine leptin (Linco, St. Charles, MO). Aliquots of 25–50 mg of adipose tissues obtained before and after the incubation were frozen in liquid nitrogen for leptin mRNA assay.

Statistical analyses. Statistica version 6.0 was used for all analyses. One-way ANOVA was used to assess differences among depots. Post hoc comparison (Newman-Keuls test) was used to compare the difference between any two depots. With pooled data from the three depots, multiple regression analysis was used to assess the effects of adipocyte volume and of mRNA levels of insulin receptor, glucocorticoid receptor, and TNF-α and of mRNA levels of insulin receptor, glucocorticoid receptor, and TNF-α on leptin mRNA levels in adipose tissue (dependent variable). To further investigate depot-of-origin effects, we included two dummy variables representing the three depots. Simple correlation analysis was used to examine the relationships between adipocyte volume and mRNA levels for leptin, insulin receptor, glucocorticoid receptor, and TNF-α and between the rates of leptin secretion and leptin mRNA levels in adipose tissues within each depot and in the pooled data. The differences in intercepts and slopes of the regression lines relating leptin mRNA and adipocyte volume among depots were tested using a multiple regression of leptin mRNA levels on adipocyte volume, depot of origin (through dummy variables), and interactions between depot of origin and adipocyte volume. The coefficients for these interaction terms represent the differences in slope among these depots. Similarly, the coefficients for the depots represent the differences in intercepts. A two-tailed P value <0.05 was considered to indicate statistical significance.

RESULTS

Depot-specific differences in adipocyte volume and in mRNA levels for leptin, insulin receptor, glucocorticoid receptor, and TNF-α. Adipocyte volume and mRNA levels of leptin, insulin receptor, glucocorticoid receptor, and TNF-α in inguinal, epididymal, and retroperitoneal adipose tissues of 10-wk-old male C57BL/6J mice (n = 15) are shown in Fig. 1. Adipocyte volumes and leptin mRNA levels were significantly lower in inguinal adipose tissue than in epididymal and retroperitoneal adipose tissues (Fig. 1). Adipocyte volumes were similar between epididymal and retroperitoneal adipose tissues, but leptin mRNA levels were higher in epididymal adipose tissue than in retroperitoneal adipose tissue, although that difference was not statistically significant. Insulin receptor mRNA levels were also lower in inguinal adipose tissue than in epididymal and retroperitoneal adipose tissue, and the difference between inguinal and retroperitoneal adipose tissues was statistically significant (P < 0.05). Glucocorticoid receptor mRNA levels were significantly higher in epididymal adipose tissue than in inguinal and retroperitoneal adipose tissues. TNF-α mRNA levels were significantly higher in inguinal adipose tissue than in epididymal and retroperitoneal adipose tissues. Average adipocyte volume was smaller in inguinal adipose tissue than in epididymal and retroperitoneal adipose tissues. Thus, unlike in rats (39), TNF-α expression levels were not positively correlated with adipocyte volumes in these mice.

Relationships of leptin mRNA levels to adipocyte volume and mRNA levels of insulin receptor and glucocorticoid receptor. Figure 2 shows correlations of adipocyte volume with mRNA levels for leptin, insulin receptor, glucocorticoid receptor, and TNF-α in inguinal, epididymal, and retroperitoneal adipose tissues. There were significant correlations between leptin mRNA levels and adipocyte volumes in each of the three depots (r = 0.63, P < 0.05 in inguinal adipose tissue; r = 0.85, P < 0.001 in epididymal adipose tissue; r = 0.83, P < 0.001 in retroperitoneal adipose tissue) (Fig. 2A). The slopes of the regression lines differed significantly between epididymal and retroperitoneal adipose tissues (P < 0.05) but not between epididymal and inguinal adipose tissues (P = 0.14) or between retroperitoneal and inguinal adipose tissues (P = 0.92). Intercepts of these regression lines were not significantly different among the three depots. These data suggest that although the major proportion of variance in leptin mRNA expression is accounted for by adipocyte volume, there are detectable depot-specific effects as well. Leptin mRNA levels and adipocyte volumes were also strongly correlated in pooled data.
from the three depots ($r = 0.80$, $P < 0.001$) (solid bold line in Fig. 2A). In contrast, there was no significant correlation between adipocyte volumes and mRNA levels for insulin receptor, glucocorticoid receptor, or TNF-$\alpha$ within each depot (Fig. 2, B–D) or in the pooled data (solid bold lines in Fig. 2, B–D). These results suggest that, unlike leptin, depot-specific differences in the expression levels of these genes were not related to adipocyte size. A significant negative correlation was found between adipocyte volumes and mRNA level for glucocorticoid receptor in retroperitoneal tissue ($r = -0.75$, $P < 0.01$). The underlying mechanism for this correlation is not clear but may be related to the anatomic proximity of retroperitoneal adipose tissue to the adrenal grand, which is the major source of circulating glucocorticoids.

Because there were significant depot-related differences in mRNA levels for insulin receptor and glucocorticoid receptor and significant differences in the slopes of the regression lines between leptin mRNA levels and adipocyte volumes in epididymal and retroperitoneal adipose tissues in these mice, we assessed relationships among adipocyte volume and mRNA levels of insulin receptor, glucocorticoid receptor, and leptin in adipocytes. TNF-$\alpha$ inhibits leptin gene expression (27, 35, 51) and was also included as an independent variable in the multiple regression. Depot of origin was also included through dummy variables (Table 2). This analysis confirmed the strong, positive correlation between adipocyte volume and leptin mRNA levels ($\beta = 0.84$, $P < 0.001$) and also showed a significant positive correlation between leptin mRNA levels and glucocorticoid receptor mRNA levels ($\beta = 0.36$, $P < 0.01$). Adipocyte volume and glucocorticoid receptor mRNA levels accounted for 64 and 9% of the variation in leptin mRNA levels in these tissues, respectively (total $R^2 = 0.80$). Insulin receptor mRNA levels ($\beta = -0.12$, $P = 0.23$) and TNF-$\alpha$ mRNA levels ($\beta = 0.18$, $P = 0.10$) showed no significant correlation with leptin mRNA levels in these mice. Including depot of origin as an independent variable in the regression analysis had no significant effect on either the standardized correlation coefficients ($\beta$ values) for adipocyte volume and glucocorticoid receptor mRNA levels or the total $R^2$ value.

Fig. 2. Correlation of adipocyte volume with mRNA levels for leptin (A), IR (B), GR (C), and TNF-$\alpha$ (D) in inguinal (○), epididymal (□), and retroperitoneal (△) adipose tissues of 10-wk-old male C57BL/6J mice (for separate depots, fine dashed or dotted lines). Regression lines (solid bold lines) and corresponding $r$ and $P$ values of the pooled data from the 3 depots are shown.
Table 2. *Multiple regression analysis of relationship of leptin mRNA levels with adipocyte volume, GR mRNA level, IR mRNA level, TNF-α mRNA level, and depot origin of adipose tissues in 10-wk-old male C57BL/6J mice*

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>R²</th>
<th>Cell volume</th>
<th>GR mRNA level</th>
<th>IR mRNA level</th>
<th>TNF-α mRNA level</th>
<th>Depot of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin mRNA level</td>
<td>0.80</td>
<td>0.84*</td>
<td>0.36*</td>
<td>-0.12 (NS)</td>
<td>0.18 (NS)</td>
<td>(NS)†</td>
</tr>
</tbody>
</table>

GR, glucocorticoid receptor; IR, insulin receptor. *P < 0.001 for the standardized correlation coefficients; NS, not significant (P > 0.05).
†R² improvement due to inclusion of 2 dummy variables representing depot of origins was not significant based on a 2-degree of freedom F-test (P = 0.965).

**DISCUSSION**

The central result of the present study is that rates of leptin mRNA expression and secretion in three anatomically distinct adipose tissue depots of lean young adult mice are primarily accounted for by adipocyte volume. The strong correlation of mRNA levels with adipocyte volume in the three depots is unique to leptin among the genes examined. Expression levels of insulin receptor, glucocorticoid receptor, and TNF-α showed
no significant correlation with adipocyte volume in the same mice. The strong correlation between leptin secretion rates and adipocyte leptin mRNA levels suggests that the synthesis and release of leptin are closely coupled and that leptin secretion is mainly regulated by transcription. The acute approximately twofold increase in leptin secretion induced by insulin points to the existence of an intracellular pool of leptin (4, 45). Our results indicate that, if leptin is stored intracellularly, the size of the intracellular pool for leptin storage is likely to be small. Although it has been suggested that leptin gene expression may be correlated with adipocyte size (23, 31, 52), this report represents the first documentation of a strong correlation between these two parameters in different fat depots without the potential confounding effects of obesity or aging. The strong correlation between leptin gene expression and adipocyte volume is consistent with the idea that leptin production by adipocytes can report their volumes. Adipose tissue mass is the product of adipocyte size and number, and hence the strong correlation of circulating leptin with total fat mass.

In addition to the predominant effect of adipocyte volume on leptin mRNA levels, which accounts for 64% of the variation in leptin mRNA levels among adipose tissue depots, glucocorticoid receptor mRNA level also has a smaller but significant positive effect on leptin mRNA levels in adipocytes, accounting for 9% of the variation in leptin mRNA levels in these mice. The significantly higher glucocorticoid receptor mRNA levels in epididymal adipose tissue than in retroperitoneal and inguinal adipose tissues apparently account for the higher leptin mRNA levels in adipocytes of similar volumes in epididymal adipose tissue than in retroperitoneal and inguinal adipose tissues (as indicated by the steeper slope of the regression line of leptin mRNA levels on adipocyte volumes in epididymal adipose tissue than in retroperitoneal and inguinal adipose tissues), constituting a depot-specific difference in leptin gene expression that is not related to adipocyte volume. These results are consistent with previous reports that glucocorticoids increase leptin gene expression in both human and rodent adipocytes and that glucocorticoid receptor mRNA levels are higher in epididymal adipose tissue than in inguinal adipose tissue in rats (4, 17, 26, 42, 45, 50).

The lack of correlation of insulin receptor mRNA levels with leptin mRNA levels suggests that the difference in the level of insulin receptor gene expression among anatomically distinct fat depots is not a major determinant of depot-specific differences in leptin gene expression in these mice. Because the mice in this study were in the postprandial state, this result is consistent with previous observations that insulin lacks direct, acute effects on leptin gene expression in the fed state (25, 47). However, differences in insulin receptor expression levels (as an indirect measure of insulin sensitivity) may have differential effects on leptin gene expression among anatomically distinct fat tissues in the fasted state. In lean rats and mice, a 24-h fast decreases leptin mRNA levels dramatically in epididymal and retroperitoneal adipose tissues but has little effect on leptin mRNA levels in inguinal adipose tissue (Ref. 59 and Zhang, unpublished results), consistent with our finding that insulin receptor mRNA levels are higher in epididymal and retroperitoneal adipose tissues than in inguinal adipose tissue in lean mice. These results suggest that leptin gene expression levels in adipocytes are proportional to adipocyte volume only in the fed state and that fasting or negative energy balance states may interrupt the relationship of adipocyte volume to leptin gene expression. In rats, fasting-induced decreases of leptin mRNA levels in epididymal and retroperitoneal adipose tissues can be largely prevented by insulin administration (47, 59), suggesting that in the fed state, insulin plays an important role in maintaining the high levels of leptin expression in epididymal and retroperitoneal adipose tissues.

There was no significant effect of depot of origin or TNF-α mRNA levels on leptin mRNA levels after adipocyte volumes and glucocorticoid receptor mRNA levels were taken into account, suggesting TNF-α and other depot-specific factors play a minimal role in depot-specific differences in leptin gene expression in these lean mice. TNF-α mRNA and activity levels are correlated with adipocyte volume and age in rats (39). Our results in young mice suggest that the increased TNF-α gene expression in these rats may be related to age or other physiological factors rather than to adipocyte volumes per se.

The mechanism underlying the strong correlation between leptin gene expression and adipocyte volume in these mice is not clear. Changes in membrane cholesterol concentration related to adipocyte size have been proposed as regulators of gene expression in adipocytes (28). Decreased membrane cholesterol concentration may result in increased sterol regulatory element binding protein 2 (SREBP-2) gene expression; both of these findings are characteristics of hypertrophic adipocytes in rodents (3, 16). SREBP-2 may, in turn, function as a coordinate regulator of gene expression in adipocytes. Manipulation of cholesterol concentrations in 3T3-L1 adipocytes does not alter leptin gene expression but does change expression patterns of several other genes, including TNF-α (28). Alternatively, leptin expression levels may not be determined by adipocyte size per se, but by rates of nutrient uptake (e.g., free fatty acid and glucose) and anabolic metabolism, which, in turn, may be correlated with adipocyte size under certain physiological conditions. As noted earlier, leptin mRNA expression is extremely sensitive to acute caloric restriction and fasting (43, 47, 59). We have also shown that leptin mRNA levels in white adipose tissues are responsive to caloric balance in rat pups (57). UDP-N-acetylglucosamine, an end product of the hexosamine biosynthetic pathway whose intracellular concentration is increased when nutrient levels are high (in anabolic state), increases leptin gene expression in muscle and adipocytes (53). In contrast to prevailing notions of declining responsiveness of adipocyte glucose uptake to insulin stimulation with in-
increasing fat cell size in older, more obese animals (5, 8, 9, 56), Hood et al. (20) reported that basal and insulin-stimulated uptake rates for glucose and palmitate were positively correlated with adipocyte size in 14-wk-old lean Zucker rats. This result is of particular interest because the effects of adipocyte size were examined in adipocytes isolated from a single epididymal fat pad, whereas many studies of the effects of adipocyte size on glucose uptake and insulin sensitivity have been done in adipocytes from individual animals that vary in age, degree of adiposity, or diet regimen. Positive correlations of adipocyte size and rates of de novo fatty acid/acylglyceride synthesis have been reported in 3-mo-old pigs (11).

In summary, we have shown that leptin gene expression is highly correlated with adipocyte volume in epididymal, inguinal, and retroperitoneal adipose tissues of fed, lean 10-wk-old male mice, and the depot-specific differences in rates of leptin gene expression are primarily accounted for by differences in adipocyte volume. Adipocyte size is a critical aspect of adipose tissue mass with regard to systemic metabolic changes because adipocyte number is relatively stable in adult humans and rodents (12, 13, 19). The strong correlation between levels of leptin mRNA expression/secretion and adipocyte size supports leptin’s importance as a signal of changes in fat mass.

**Perspectives**

The so-called “lipostatic” hypothesis postulated primarily by Kennedy (24) and Hervey (18) nearly 50 yr ago suggested that somatic fat stores somehow signal the brain regarding the status of systemic energy balance. Hirsch (19) subsequently emphasized that total fat mass was the product of cell number and cell size and that cell size reflects short- and intermediate-term changes in total fat mass. Therefore, whatever signal is being provided by fat stores, it was predicted that it would be proportionate to fat cell volume. Insulin, in fact, has this characteristic and can provide signals to the central nervous system (hypothalamus) proportional to fat cell volume (55). The parabiosis experiments of Coleman (6) suggested that the ob gene product (leptin) might also be a humoral signal proportional to fat mass. The cloning and characterization of the leptin gene confirmed that inference (14). In the studies reported here, leptin gene expression and protein production are shown to closely obey the predicted relationship to adipocyte volume across anatomically distinct fat depots in ad libitum-fed nonobese mice. The responsiveness of leptin gene expression to caloric balance in adipocytes and to adipocyte volume enables this secreted molecule to provide a precise measure of the size of adipose tissue energy stores as well as early warning of potential deficits in these stores. This demonstration provides a critical link between the molecular genetics of energy homeostasis and its realization in systemic physiology. It is important to note that the molecular bases for the proportionality of circulating insulin and leptin to fat cell volume are unknown. The mechanisms are likely to be quite different, and answers to these questions would provide deep insights into the cell biology of energy homeostasis.

We thank Q. Li and M. Su for assistance in preparing and staining of adipose tissue sections, and Dr. Y. Yu for helpful discussion. This work was supported in part by the Career Development Award from the American Diabetes Association (to Y. Zhang) and by National Institute of Diabetes and Digestive and Kidney Diseases Grants R01-DK-52431 and 2-P-30-DK-26687 (to R. L. Leibel).

**REFERENCES**


