Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone

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1Department of Nutritional Sciences, Rutgers, The State University of New Jersey, and 2Department of Surgery, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey 08901; and 3Laboratory of Human Behavior and Metabolism, The Rockefeller University, New York, New York 10021

Russell, C. D., R. N. Petersen, S. P. Rao, M. R. Ricci, A. Prasad, Y. Zhang, R. E. Brolin, and S. K. Fried. Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E507–E515, 1998.—We investigated the in vitro regulation of leptin expression in adipose tissue from severely obese women and men before and after culture with insulin (7 nM) and/or dexamethasone (25 nM). Leptin mRNA and leptin secretion were two- to threefold higher in subcutaneous vs. omental adipose tissue before culture. Dexamethasone transiently increased leptin mRNA approximately twofold in both depots after 1 day of culture (P < 0.01 vs. basal [no hormone control]), but leptin secretion was only increased in omental adipose tissue (P < 0.005 vs. basal). Insulin did not increase leptin mRNA in either depot but increased leptin secretion ~1.5- to 3-fold in subcutaneous tissue throughout 7 days of culture (P < 0.05 vs. basal). The combination of insulin and dexamethasone increased leptin mRNA and leptin secretion approximately two- to threefold in both depots at day 1 (P < 0.005 vs. basal or insulin) and maintained leptin expression throughout 7 days of culture. We conclude that insulin and glucocorticoid have depot-specific effects and function synergistically as long-term regulators of leptin expression in omental and subcutaneous adipose tissue from obese subjects.

glucocorticoids; obesity; organ culture

Leptin is a recently discovered adipocyte hormone thought to communicate the status of adipose stores to central systems regulating food intake and energy expenditure. Circulating levels of leptin correlate well with the level of adiposity in rodents and humans (8, 22). However, serum leptin levels can change without concomitant changes in adiposity. For instance, serum leptin levels decline significantly after 12 h of fasting in lean and obese humans and are restored after refeeding (17). Massive overfeeding (120 kcal/kg) for as little as 5 h increases serum leptin 40% in healthy individuals (19). The hormones or factors mediating these effects are not yet clear.

Many groups have investigated the hypothesis that nutritional regulation of serum leptin (occurring in advance of body weight changes) is mediated by changes in serum insulin levels. Results of numerous studies indicate that insulin effects on leptin require several hours at least. In vivo, the earliest increases in leptin appeared only after 4 h of supraphysiological insulin infusion (35). Yet other studies report no increases before 24 (1) or 48 h (16) of clamped hyperinsulinemia within the physiological range. Although serum leptin tends to correlate with fasting insulin levels (2), careful comparison between the time course of changes in serum leptin and insulin suggests that acute effects of endogenous or exogenous insulin do not fully explain changes in leptin observed in over- and underfeeding experiments (19, 31). Therefore, chronic, rather than acute, insulin exposure may be more significant to the regulation of leptin expression.

Few studies have examined the in vitro regulation of leptin by insulin in human fat cells. In primary cultures of collagenase-isolated human adipocytes from a small number of subjects, insulin increased leptin mRNA at 72 h, followed by an increase in leptin in the culture medium after 96 h (16). In cultures of newly differentiated human adipocytes, insulin had only a small stimulatory effect on leptin production at 24 h (36).

Glucocorticoids have been shown to increase leptin levels more consistently than insulin. In vivo, we (28) and others (18, 20, 25) have demonstrated that oral glucocorticoids increase leptin mRNA and serum leptin approximately twofold 24–48 h after administration. One study found an increase after only 8 h (25). Although these increases in leptin expression in vivo could be partly mediated by the glucocorticoid-induced increase in serum insulin, they do not appear to be completely dependent on it (20). Incubation of rat (10) or human adipocytes with dexamethasone increases leptin mRNA and/or secretion. Thus results of in vitro studies argue for a direct effect of glucocorticoid as well.

Further complicating in vivo study of leptin regulation is the fact that different adipose depots may not contribute proportionately to serum leptin, since regional differences in leptin mRNA abundance have been noted (24, 26). In vivo, visceral fat mass does not predict serum leptin levels as strongly as total fat mass (34). Moreover, different adipose depots may exhibit different levels of sensitivity and/or responsiveness to hormones, such as insulin and glucocorticoid (11, 37). It is therefore important to study leptin regulation in both intra-abdominal and subcutaneous depots.

Insulin and glucocorticoid are known to synergize in regulating the expression of adipocyte genes, such as lipoprotein lipase (LPL) (11) and GLUT-4 (12). Reports of the effects of this combination of hormones on leptin expression conflict. In isolated human (7) or rat (29)
adipocytes, culture with insulin appeared to inhibit dexamethasone-induced increases in leptin mRNA and secretion. Wabitsch et al. (36), however, showed that dexamethasone potentiates insulin-stimulated increases in leptin secretion in newly differentiated primary cultures of human adipocytes. Insulin and glucocorticoid are elevated in response to a meal (4) and may facilitate the development or maintenance of the obese state (23). The role of these hormones in regulating leptin expression needs to be clarified.

Thus, in the present work, we studied the regulation of leptin expression by insulin and glucocorticoid in vitro using fragments of omental and subcutaneous adipose tissue from obese individuals. The time course of hormonal effects on leptin mRNA and secretion was assessed by maintaining the tissue for up to 1 wk in a well-characterized organ-culture system. We report depot differences in the regulation of leptin expression and show that a combination of insulin and dexamethasone best maintained leptin expression in both depots over a full week of culture.

**METHODS**

**Subjects.** Adipose tissue from the omental (OM) and abdominal subcutaneous (SQ) depots of 60 severely obese individuals (36 premenopausal females and 24 males) was obtained during elective surgery (gastropasty) at Robert Wood Johnson University Hospital. Table 1 shows subject characteristics. As indicated by their body mass indexes, most of the subjects in this study were severely obese. Some subjects had lost weight due to previous obesity surgery, but all were obese and weight stable for at least 1 mo before surgery. No differences due to previous weight loss were observed; therefore, all data are not due to previous obesity surgery, but all were obese and weight stable for at least 1 mo before surgery. No differences due to previous weight loss were observed; therefore, all data are pooled. Other than obesity, subjects were free of overt metabolic diseases (e.g., diabetes and cancer) and were not taking medications known to interfere with metabolism. Several subjects were taking antihypertensive medication (Ca++ channel blockers or angiotensin-converting enzyme inhibitors), but results from this group were similar to the rest and so the data were included.

**Tissue preparation.** At surgery, aliquots of fat from OM and SQ adipose tissue were immediately frozen in dry ice and subsequently stored at −80°C for determination of initial (preculture) levels of mRNA. Other aliquots were placed into tubes containing medium 199 (GIBCO BRL), finely minced, and placed in organ culture, as previously described (11, 33). Briefly, tissue fragments were cultured in serum-free medium 199 alone (basal) or in the presence of 7 nM insulin (Humulin, Lilly, Indianapolis, IN), 25 nM dexamethasone, or the combination of both. These hormone concentrations were chosen empirically because they produced maximal effects on levels of LPL mRNA and LPL activity (11). Organ-culture medium containing fresh hormones was replenished every other day. At day 7, samples of tissue were assayed for heparin-releasable LPL activity to verify the hormonal responsiveness of the tissue (11).

**Determination of fat cell size.** Mean fat cell weight was determined by Coulter counting of osmium-fixed fragments of adipose tissue as described by Hirsch and Gallian (14).

**Determination of leptin mRNA levels.** Total RNA was extracted by a modified method of Chomczynski and Sacchi (5), gel electrophoresed (1% agarose, 37% formaldehyde) to separate RNA species by size, and then transferred onto a nylon membrane (Genescreen, Du Pont-NEN, Boston, MA) by electrophobting, as previously described (11). These Northern blots were sequentially probed with 32P-labeled cDNAs (random primer, BRL) for human leptin (1.0-kb cDNA fragment excised with EcoR I, courtesy of J. M. Friedman), 28S rRNA, and, in some cases, human LPL. After washing (2 times with 1% SDS–1× sodium chloride–sodium citrate buffer (SSC) and once with 1% SDS–0.1× SSC at 55°C), blots were exposed to X-ray film. Samples for analysis of leptin levels 1) before and after culture and 2) between OM and SQ adipose tissue from the same patient were always placed on the same gel to facilitate comparisons. Bands corresponding to human leptin (4.5 kb) and ethidium bromide-stained 28S RNA (total RNA) were scanned using a hand-held or flatbed scanner, and the resulting images were analyzed using UN-SCAN-IT software (Silk Scientific, Orem, UT). The ratios of absolute pixel intensities of the leptin mRNA and ethidium bromide-stained 28S RNA bands were calculated (leptin/28S). After it was determined that photographs of ethidium bromide-stained 28S bands were similar to autoradiographs from cDNA-probed 28S rRNA, the former were used as a loading control because band intensities were more easily assessed.

**Reversibility of hormonal effects on leptin mRNA.** To determine whether leptin mRNA expression was altered by abrupt changes in hormonal milieu, hormones were withdrawn from tissue cultured for 1 wk with the combination of insulin and dexamethasone. In other experiments, the combination of insulin and dexamethasone was added to tissue that had been cultured for 1 wk with only insulin. In a subset of the latter experiments, the transcription inhibitor 5,6-dichlorobenzimidazole riboside (DRB) (32) was added for a final concentration of 200 µM at the same time that the medium was changed. Total RNA was extracted, and leptin message was analyzed after 24 h, as described above. Parallel cultures of tissue were maintained in the original hormonal milieu to serve as controls.

**Measurement of leptin protein.** The amount of leptin in aliquots of culture medium was measured for a subset of obese subjects used for leptin mRNA determination and having similar body mass indexes and fat cell sizes. Leptin protein was determined by RIA using a kit from Linco (St. Charles, MO). Samples of culture medium were taken at indicated time points during culture. Data are presented in nanograms of leptin per gram of adipose tissue per 24 h of culture. In a subset of patients, adipocytes were isolated by collagenase digestion, as previously described (11). Leptin secretion was then measured from both tissue fragments and isolated adipocytes during an acute incubation as follows. Adipose tissue (−100 mg) or adipocytes (−5 × 10⁶) were incubated in medium 199 containing 1% BSA for 3 h at 37°C with shaking (55 cycles/min). Phenylisopropyladenosine (100 nM) was included in the medium for digestion, cell washing, and incubations of adipocytes to reduce cell fragility. Acute

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>BMI, kg/m²</th>
<th>Fat Cell Size, µg lipid/cell</th>
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<tr>
<td>Females</td>
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<tr>
<td>40 – 2</td>
<td>48 ± 2</td>
<td>OM 0.61 ± 0.05 SQ 0.71 ± 0.05</td>
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<td>(20 – 57)</td>
<td>(29 – 68)</td>
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<tr>
<td>Males</td>
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<tr>
<td>44 ± 2</td>
<td>47 ± 2</td>
<td>OM 0.68 ± 0.06 SQ 0.77 ± 0.06</td>
</tr>
<tr>
<td>(33 – 60)</td>
<td>(28 – 66)</td>
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Values are means ± SE with ranges in parentheses; n = 36 for females and n = 24 for males. OM, omental; SQ, subcutaneous; BMI, body mass index.
incubation values are presented as nanograms of leptin released per 10⁶ adipocytes over 3 h.

Data analysis. All data are presented as means ± SE. Statistical comparisons of hormonal effects and depot differences were made by ANOVA of log-transformed values (Systat Software, Evanston, IL). Gender was used as a grouping factor, and hormone, depot, and time were considered as repeated measures. The relative magnitude of hormone or depot effects was not dependent on gender; therefore, all data were pooled for subsequent statistical analysis. In the analysis of leptin mRNA levels after culture, it was not always possible to compare absolute levels of leptin mRNA between different subjects, since they were not placed on the same gels. We therefore compared the relative hormonal effects within patients. Where indicated, samples of RNA from OM and SQ adipose tissue were analyzed on the same gels to directly compare these two depots. For analysis of levels of immunoreactive leptin protein or relative leptin mRNA abundance, when overall F values for main effects or interactions of hormones were statistically significant by F test, individual means were compared, followed by post hoc paired t-tests on log-transformed data. P < 0.05 was considered statistically significant.

RESULTS

Leptin mRNA levels in adipose tissue before culture. Leptin mRNA was found only in the adipocytes of adipose tissue and not in the stromal-vascular fraction (data not shown), as also noted by others (24). In adipose tissue analyzed immediately after excision from these morbidly obese individuals, the level of leptin mRNA in the abdominal SQ depot was higher than in the OM depot (P < 0.001, within patients; Figs. 1A and 4A), and there was no interaction of this effect with gender. The average difference was approximately threefold between OM and SQ depots in both men and women (Fig. 1A) and did not appear to relate to the size of the fat cells, which was on average similar (Table 1).

To determine whether the depot difference in leptin mRNA was accompanied by a difference in leptin secretion, adipose tissue fragments or isolated adipocytes were incubated for 3 h. The amount of leptin secreted was approximately twofold higher in incubations of SQ vs. OM adipose tissue or isolated adipocytes (P < 0.05 within subjects; n = 7; Fig. 1B).

To directly compare the relative abundance of leptin mRNA between different subjects, samples of RNA from OM and SQ adipose tissues of a subset of women and men were analyzed on the same gel. There were no gender-related differences in leptin mRNA levels in this severely obese group of patients (OM: women, 0.22 ± 0.03 (n = 5) vs. men, 0.32 ± 0.02 (n = 8); SQ: women, 0.74 ± 0.05 (n = 8) vs. men, 0.58 ± 0.04 (n = 9); not significant (NS) by independent t-test, men vs. women in each depot).

Hormonal effects on leptin expression in cultured SQ adipose tissue. We examined leptin mRNA and secretion in SQ adipose tissue cultured for 1 day in serum-free medium (basal) containing 7 nM insulin or 25 nM dexamethasone, or a combination of both. The relative abundance of leptin mRNA in tissues cultured with dexamethasone or with the combination of insulin plus dexamethasone was significantly higher than in those without hormones (basal) or with insulin alone (P < 0.05, n = 7; Fig. 2A). Culture with insulin alone did not, on average, affect leptin mRNA levels in this depot.

We also measured the immunoreactive leptin accumulating in the culture medium of SQ adipose tissue after 1 day (24 h). Similar to leptin mRNA levels, the leptin in the medium of tissue cultured with the combination of insulin and dexamethasone was higher than in all other culture conditions (Fig. 2C) and was significantly higher than basal after only 5 h of culture (n = 6, P < 0.05, data not shown). Dexamethasone alone did not significantly increase leptin in the medium. Culture of SQ tissue with insulin, however, increased leptin in the medium by ~50% (P < 0.005 vs. basal) despite the lack of significant effect on leptin mRNA levels.

To examine the long-term effects of these hormones, we analyzed the expression of leptin in subcutaneous

Fig. 1. A: relative abundance of leptin mRNA in omental and subcutaneous adipose tissue before culture. Total RNA was isolated from omental (OM; shaded bars) and abdominal subcutaneous (SQ; open bars) adipose tissues (quick-frozen at time of surgery) of obese subjects. Northern blots were probed with 32P-labeled human leptin cDNA. Ethidium bromide (EtBr) staining of 28S RNA band on gels served as a loading control, as described in METHODS. Ratios of pixel intensities of leptin mRNA on scanned autoradiographs to EtBr-stained 28S bands (leptin/28S) are presented as means ± SE; n = 31. Depot differences are statistically significant (P < 0.05) in both males (n = 14) and females (n = 17) or pooled data (P < 0.001), by paired t-test of log-transformed values. B: leptin secretion before culture. Immunoreactive leptin secreted from adipose tissue fragments and isolated adipocytes of 7 subjects (4 females, 3 males) during a 3-h incubation was determined (see METHODS). Data are expressed as nanograms of leptin per 10⁶ adipocytes. *P < 0.05.
tissue within each hormonal condition over 1 wk of culture. Leptin mRNA levels declined between days 1 and 7 of culture without hormones or with either insulin or dexamethasone alone (P < 0.05), but the combination of insulin plus dexamethasone maintained leptin mRNA at levels that were 84 ± 18% of preculture values in SQ tissue. Changes in leptin secretion within a hormonal treatment over 1 wk in culture were also assessed. Medium was sampled 24 h after renewal of the culture medium; thus day 3 and 7 values represent leptin accumulating between days 2–3 and 6–7 of culture, respectively. In cultures containing insulin or insulin plus dexamethasone, leptin levels in the medium on days 3 and 7 were similar to day 1 values; there was no significant decline over time (NS by ANOVA). In cultures containing either no hormones or dexamethasone alone, leptin in the medium was significantly lower on days 3 and 7 vs. day 1 (P < 0.005 by ANOVA; Fig. 2C).}

When leptin expression was compared between treatments on day 7 of culture, levels of leptin mRNA (Fig. 2B) as well as medium leptin (Fig. 2C) were two- to threefold higher in tissue cultured with the combination of insulin plus dexamethasone compared with
basal and insulin or dexamethasone alone. These effects of insulin and dexamethasone were synergistic (insulin × dexamethasone interaction was \( P < 0.05 \) by ANOVA). Culture with insulin alone did not increase leptin mRNA over basal levels (Fig. 2B). Although leptin mRNA was not increased by insulin, this was not due to a general insensitivity of SQ tissue to insulin effects. Insulin significantly increased LPL activity and LPL mRNA levels in SQ adipose fragments but not in the less insulin-sensitive OM tissue, as previously reported (Ref. 11; data not shown). Insulin significantly increased leptin in the culture medium relative to basal and dexamethasone (Fig. 2C). The high leptin mRNA expression in tissue cultured with dexamethasone observed on day 1 appears to be transient, since leptin mRNA and secretion were no higher than basal levels on day 7 of this treatment.

Hormonal effects on leptin expression in cultured OM adipose tissue. Similar to results from SQ depot, 1 day of culture with dexamethasone or with insulin plus dexamethasone increased leptin mRNA in OM cultures (each \( P < 0.01 \) vs. basal, \( n = 8 \); Fig. 3A). Leptin accumulation in the medium over this first 24 h of culture was also higher under these two conditions compared with basal or insulin (\( P < 0.001 \)), but at 5 h of culture, no hormonal effects were significant (data not shown). In this depot, culture with insulin alone significantly decreased leptin mRNA, but not leptin secreted into the culture media, relative to the basal or dexamethasone conditions (\( P < 0.05 \); Fig. 3A and C).

When we examined leptin expression within hormonal conditions over chronic culture, the combination of insulin plus dexamethasone maintained OM leptin mRNA at 70 ± 11% of preculture levels. In contrast, leptin message levels declined when tissue was cultured with either insulin or dexamethasone alone (OM: \( n = 11 \); Fig. 4, A and C). The time course of leptin accumulating in the culture medium was analyzed as described for the SQ depot. Culture with the combination of insulin and dexamethasone maintained levels of secreted leptin in the culture medium (measured on days 3 and 7) at values similar to those on day 1 (NS by ANOVA; Fig. 3C), just as in the SQ depot. The effect of insulin plus dexamethasone was synergistic at days 3 and 7 (\( P < 0.05 \) by ANOVA). Leptin in cultures containing insulin remained similar to day 1 levels. However, leptin in the media of cultures containing dexamethasone alone dropped significantly by day 3 and through day 7 of culture compared with the day 1 values (\( P < 0.01 \) by ANOVA; Fig. 3C).

![Image](https://placekitten.com/48/58/410x434)

**Fig. 3.** Effects of insulin and/or dexamethasone on relative abundance of OM leptin mRNA and leptin accumulating in culture medium. OM adipose tissue from obese subjects was cultured for 1 (A) or 7 days (B) as described in legend for Fig. 2. Ratios of pixel intensities of bands corresponding to leptin mRNA and EtBr-stained 28S RNA (leptin/28S) are shown as means ± SE. Bars with different superscripts are significantly different (at least \( P < 0.05 \) of logged-transformed values) when hormonal effects are compared within subjects. Day 1: \( n = 8 \) (6 females, 2 males); day 7: \( n = 16 \) (12 females, 4 males). Typical Northern blots showing leptin mRNA band are shown above graphs. EtBr staining of total RNA is shown to indicate loading. C: leptin accumulating in culture medium was measured as described in legend for Fig. 2. Data are means ± SE of 6 subjects (5 females, 1 male), in which samples are matched to compare effect of time. \# \( P < 0.05 \) compared with day 1). Bars with different letters are significantly different from each other within each day (at least \( P < 0.05 \) by paired t-test). Note that in this time-matched group (\( n = 6 \)), I vs. ID on day 7 was \( P = 0.066 \) but, in a slightly larger group (\( n = 8 \), matched for hormonal condition on day 7 (\( P = 0.026 \)).
When leptin expression was compared between treatments on day 7 of culture, we found that the levels of leptin mRNA were approximately two- to threefold higher after culture with the combination of insulin plus dexamethasone compared with basal ($P < 0.05$), insulin ($P < 0.001$), or dexamethasone alone ($P < 0.001$, $n = 16$; Fig. 3B). A similar pattern was seen in leptin secretion at day 7, when leptin in the medium was significantly higher in cultures containing the combination of these hormones (insulin + dexamethasone) vs. basal ($P < 0.01$), dexamethasone ($P < 0.005$), or insulin ($P = 0.066$ in a time-matched subset, see Fig. 3C; but $P = 0.02$ with $n = 8$, data not shown). Neither insulin nor dexamethasone alone increased leptin mRNA or leptin in the medium over basal values on day 7.

**Depot differences.** The depot difference in leptin mRNA abundance seen before culture (SQ > OM) persisted after 1 and 7 days of culture, but only in tissue cultured with the combination of insulin plus dexamethasone ($P < 0.05$; Figs. 1A and 4). However, the depot difference in leptin secretion from adipose tissue or isolated adipocytes incubated before culture was no longer apparent at 1, 3, or 7 days of culture with insulin plus dexamethasone. The amount of leptin in the media of SQ and OM adipose tissue was similar after culture with the combination of these two hormones (Figs. 2C and 3C).

**Responsiveness to changes in hormonal milieu after prolonged culture.** To determine whether leptin mRNA expression was responsive to abrupt changes in hormonal milieu even after prolonged culture, adipose tissue that had been cultured for 7 days with insulin plus dexamethasone was switched into medium without any hormones (basal). After 24 h, leptin mRNA was decreased to $26 \pm 7\%$ of control (tissue maintained in insulin + dexamethasone) in all samples [$n = 8$ (5 OM, 3 SQ), $P < 0.05$; Fig. 5]. In another set of experiments, we assessed whether leptin expression could still be upregulated after extended culture. Tissue that had been cultured with only insulin for 7 days was switched into medium containing both insulin and dexamethasone. At 24 h, leptin mRNA levels had increased to $273 \pm 14\%$ of control (tissue maintained in insulin + dexamethasone) in all samples [$n = 5$ (2 OM, 3 SQ), $P < 0.05$; Fig. 5]. When, in a subset of these experiments, the transcription inhibitor DRB was added as well as insulin plus dexamethasone, the increase was clearly blunted [DRB: $16 \pm 4\%$ vs. control: $349 \pm 16\%$; $n = 3$ (2 SQ, 1 OM); Fig. 5].
We have demonstrated that insulin and glucocorticoid function synergistically as long-term regulators of leptin expression in vitro in both omental and subcutaneous adipose tissue of severely obese subjects. Throughout 7 days of culture, the combination of these hormones maintained levels of leptin mRNA and rates of leptin secretion at values close to those found in freshly obtained adipose tissue. These results are consistent with the hypothesis that the chronic hyperinsulinemia and increased cortisol turnover typical of obese individuals (23) are important in maintaining high leptin expression.

Because we conducted these experiments exclusively on adipose tissue from obese subjects, it is not clear whether similar regulation occurs in adipose tissue from leaner individuals. However, prolonged (72 h) hyperinsulinemia in vivo increases serum leptin in normal-weight subjects (1, 16, 35). In addition, we (28) and others (9, 18) have shown that oral administration of a moderate dose of dexamethasone increases leptin mRNA levels and serum leptin within 48 h in both lean and obese subjects. The present data show that insulin and dexamethasone directly affect leptin mRNA levels in adipose tissue from obese subjects in vitro.

We found that the interaction between insulin and dexamethasone became synergistic after prolonged (7 day) culture. Our results agree well with those of Wabitsch et al. (36), who reported that cortisol potentiated an insulin-stimulated increase in leptin expression in vitro differentiated human adipocytes derived from the stromal fraction of mammary adipose tissue of moderately obese women. Other groups that have failed to observe synergy between these two hormones have used shorter incubation times and/or used a high concentration of serum (~10%) in their culture or incubation medium (7, 13, 29). Wabitsch et al. included only 0.2% serum, and we used serum-free culture. Considine et al. (7) found that incubation with insulin inhibited a dexamethasone-stimulated increase in leptin expression in human fat cells (10% serum present). However, this group's subsequent in vivo experiments, in which subjects were treated with a high dose of oral dexamethasone (10 mg/day) while undergoing a hyperinsulinemic clamp, failed to mimic the in vitro inhibition (18). Potentially, additional factors and/or hormones in serum have some role in modulating the regulation of leptin expression by insulin and glucocorticoid.

We report that leptin secretion from subcutaneous adipose tissue is significantly increased by the combination of insulin and dexamethasone after only 5 h of culture and nearly doubles by 24 h of culture. This time course concurs with the in vivo data of Ultriainen et al. (35), who found that high doses of insulin increased leptin by 4 h. Increases in serum leptin have been found only 8 h after administration of oral dexamethasone (25). Increases in serum leptin due to overfeeding are observed as soon as 5 h (19). Our data therefore suggest that the effects of insulin and cortisol in vivo may underlie these swift responses of serum leptin.

Insulin had depot-specific effects on leptin expression. In subcutaneous tissue, the relative abundance of leptin mRNA was not significantly increased by insulin at any time point, but some samples did exhibit small increases or decreases in leptin mRNA (cf. Figs. 2A and 4A). However, insulin consistently increased leptin secretion from subcutaneous adipose tissue throughout the week of culture, even in the face of decreased leptin mRNA in some subjects. This effect of insulin on secreted leptin may be specific, such as a posttranscriptional regulation of leptin synthesis and/or secretion. Alternatively, insulin may cause a general increase in total cellular protein synthesis (11) that could contribute to higher leptin secretion. Elucidation of this mechanism requires further investigation. In contrast to our results, another group culturing isolated fat cells with insulin did not see a rise in secreted leptin until after 96 h (16). Because adipose tissue fragments were used in our study, paracrine mediation of hormonal effects involving nonadipocytes cannot be ruled out. Studies using well-characterized culture of isolated human adipocytes are necessary to resolve this issue.

In contrast to results from subcutaneous tissue, we found that omental leptin mRNA was significantly decreased (relative to basal levels) after 1 day of culture with insulin. In previous work, we have noted that omental adipose tissue is less responsive than subcutaneous tissue to insulin-stimulated increases in LPL expression, but an actual decrease in leptin mRNA with insulin was unexpected. Omental leptin secretion, however, was not significantly different between insulin and basal conditions.

This is the first report of glucocorticoid effects on leptin expression in intra-abdominal adipose tissue (see NOTE ADDED IN PROOF). Dexamethasone increased leptin mRNA in tissue from both omental and subcutaneous depots by 24 h, but an increase in secretion was
significant only in the omental depot. Similarly, we have previously noted that LPL expression in omental adipose tissue is more responsive to dexamethasone than subcutaneous tissue (11), probably due to a higher relative density of glucocorticoid receptors in the omental depot (3).

Hormonal effects were reversible even after prolonged culture. In agreement with others (36), withdrawal of hormones decreased leptin expression. The increase in leptin mRNA expression observed 24 h after adding dexamethasone to insulin-treated cultures probably has a transcriptional component, since it was blocked by simultaneous addition of the transcription inhibitor DRB. The leptin (ob) gene has been found to contain a number of glucocorticoid response elements (11a), so that a transcriptional effect of dexamethasone is not surprising. Taken together, these data indicate that leptin mRNA levels in human adipose tissue respond relatively quickly to abrupt changes in the hormonal milieu, in part via transcriptional mechanisms.

The rates of leptin secretion from adipose tissue fragments during organ culture are 10-fold higher than those observed in newly differentiated adipocytes (~20 ng vs. ~2 ng·(10^6 adipocytes)^{-1}·h^{-1}) (36). The values that we obtained are close to those reported by Klein et al. (15) for the rate of leptin secretion from human abdominal adipose tissue as determined by an arteriovenous difference technique. It thus appears that organ culture is a physiologically relevant system for studies of the factors that regulate leptin production in human adipose tissue over the long term, just as it has been for studies of other genes and metabolic processes.

As others have reported (24, 26), subcutaneous adipose tissue exhibits higher leptin mRNA expression than omental tissue. In vivo, serum leptin levels correlate poorly with the amount of visceral fat mass in obese and normal-weight subjects (34). Accordingly, we found that the rate of leptin secretion from omental adipose tissue was only half that of subcutaneous. Visceral fat depots may not contribute as substantially to serum leptin levels and consequently to central feedback mechanisms, potentially permitting accumulation of intra-abdominal adipose mass. The depot difference in leptin secretion implicates regional adiposity as an important determinant of serum leptin levels, at least in the obese.

Absolute differences between women and men in levels of serum leptin (27) or leptin mRNA (21) have also been noted. We directly compared omental and subcutaneous leptin mRNA levels in a small subset of subjects but found no obvious differences. Nor did we find a gender difference in the depot ratio of leptin mRNA, in contrast to Montague et al. (26), who, using tissue from mildly obese or nonobese subjects, found that women had a higher ratio of subcutaneous to omental leptin mRNA abundance. Severely obese individuals may not show the same magnitude of gender difference in leptin mRNA expression as leaner subjects, but this requires further study.

We did not find a difference between obese men and women in the responsiveness to insulin and/or glucocorticoid effects in adipose tissue. These in vitro results are consistent with in vivo findings that administration of insulin or oral dexamethasone has similar effects in men and women (9, 28). If confirmed in leaner subjects, these data suggest that an altered responsiveness to these hormones is not a mechanism contributing to the higher leptin expression observed in women in vivo (27). In our study, hormone concentrations were chosen to provide maximal responses. Preliminary studies show that, in agreement with our prior work with LPL (11), half-maximal responses of leptin expression are achieved at physiologically relevant concentrations (0.7 nM insulin, 2.5 nM dexamethasone). Whether hormonal sensitivity of leptin expression differs between women and men or lean and obese subjects remains to be clarified.

In conclusion, we have provided evidence that, in adipose tissue from obese subjects, insulin and glucocorticoid are long-term regulators of leptin expression. These data suggest that the hyperinsulinemia and increased cortisol turnover associated with the obese state contribute to the maintenance of high leptin expression in these individuals. Given the time course of response, it is conceivable that these hormones play a role in mediating nutritional effects on serum leptin in vivo. Furthermore, depot-specific regulation of leptin expression by insulin and dexamethasone may underlie depot differences in leptin secretion and therefore their contribution to serum leptin concentrations.

NOTE ADDED IN PROOF


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