

Steroid-Dependent Up-Regulation of Adipose Leptin Secretion In Vitro During Pregnancy in Mice¹

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ABSTRACT

Circulating leptin levels are elevated during the later stages of pregnancy in mammals, suggesting that maternal leptin may play a role in maintenance of pregnancy and/or preparation for parturition and lactation. The regulation and source of circulating leptin during pregnancy remains undetermined, but leptin mRNA levels increase in adipose tissue during this time in some species. Considerable controversy exists whether placenta is also a leptin-secreting tissue during pregnancy. Here, we directly demonstrate that leptin secretion rates from mouse adipose tissue *in vitro* are decreased during early pregnancy and up-regulated during late pregnancy and lactation. Changes in leptin secretion rates *in vitro* paralleled those of circulating leptin *in vivo* during gestation. Subcutaneous implants of estradiol or corticosterone into lactating mice for 48 h stimulated adipose leptin secretion rates *in vitro* to the level of that in pregnant mice. However, corticosterone, but not estradiol, increased leptin secretion when added to isolated adipose tissue *in vitro*. Placentae obtained at two stages of pregnancy did not secrete leptin *in vitro*, either when acutely isolated or when dissociated into cells for long-term cultures. Placental tissue (or cells) secreted progesterone, however, demonstrating placental viability. We conclude that hyperleptinemia during late pregnancy in mice primarily results from corticosterone-dependent up-regulation of leptin secretion from adipose tissue, and that the placenta does not contribute to leptin secretion. The initial decrease in leptin secretory rates from adipose tissue during early pregnancy may facilitate energy storage for the subsequent, increased metabolic demands of later pregnancy and lactation.

adrenal cortex, corticosterone, estradiol, lactation, leptin, placenta, pregnancy

INTRODUCTION

Leptin is an important feedback controller of energy balance that acts to increase metabolism and depress appetite [1]. It is secreted by adipose cells, and its circulating concentration normally correlates with body adiposity [2]. During mid to late pregnancy, however, circulating leptin levels increase in rats [3, 4], mice [5, 6], bats [7, 8], and humans

[9–12], but they do not consistently correlate with adiposity during this time. This has produced suggestions that leptin may exert nonmetabolic actions during pregnancy, such as stimulation of prolactin secretion [13], fetal growth and development [14], or placental function [15]. Thus, because of its potential roles in pregnancy and lactation, coupled with the exceptional metabolic demands of late pregnancy and lactation, an understanding of the regulation of leptin secretion during these critical periods should be established.

The source of elevated leptin during late pregnancy is unknown. The lack of a consistent correlation between circulating leptin and body adiposity in pregnant animals [9], however, suggests that leptin is secreted during pregnancy from a nonadipose site (or sites). Consistent with this hypothesis, human placentae and cell lines derived from human choriocarcinomas express leptin mRNA at comparable or greater levels than adipose tissue, and they also produce leptin protein [10, 16–19]. In rodents, however, conflicting results have been reported. Kawai et al. [3] reported that leptin mRNA in pregnant rats was expressed in adipose but not in placental tissue. Other investigators have observed low levels of leptin mRNA in rat placenta [4, 20], which in one case [4] increased as pregnancy progressed. In mice, leptin protein content in the placenta is high [6], but whether leptin mRNA is expressed in mouse placenta is equivocal [5, 21]. Thus, whether the placenta contributes to hyperleptinemia of pregnancy in rodents remains unresolved.

A clear increase in adipose leptin mRNA, however, has been observed during pregnancy in mice [6], suggesting that adipose tissue may be a major source of elevated serum leptin during pregnancy. However, this has not been demonstrated by direct determination of leptin secretion rates from adipose tissue as a function of reproductive status.

Finally, the factors responsible for the progressive increase in serum leptin among pregnant mammals are unknown. The increased circulating leptin levels may be hormone-dependent, because at least two of the hormones associated with pregnancy (i.e., glucocorticoids and estradiol) up-regulate leptin mRNA in adipose tissue [22, 23]. However, the effect of physiological *in vivo* elevations of these hormones on the rate of leptin secretion from adipose tissue *in vitro* has not been determined in pregnant or lactating animals.

Thus, because of the conflicting results for leptin gene expression and the lack of experimental data demonstrating that changes in leptin gene expression are coupled with changes in leptin secretion, this study was designed to: 1) directly determine, to our knowledge for the first time, whether adipose leptin secretion is up-regulated during pregnancy in mice; 2) determine whether leptin is secreted from mouse placenta; and 3) determine whether the gonadal and adrenal steroid hormones associated with pregnancy contribute to increased leptin secretion during pregnancy.

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MATERIALS AND METHODS

Animals

Timed-pregnant Swiss-Webster mice (Charles River Laboratories, Raleigh, NC) were individually housed (lights-on from 0700–1900 h) with water and commercial laboratory chow available ad libitum. On the day of an experiment, food was withdrawn at 0900 h, which was 2–3 h before the animals were killed by exposure to carbon dioxide. Trunk blood and omental fat were obtained from mice on Days 7, 14, and 20 of pregnancy and on Days 1, 2, and 5 of lactation. These days were chosen to approximate the end of each trimester as well as the early and later stages of lactation. In previous studies in bats, we found that the plasma leptin level declined after 48 h of lactation and reached a stable baseline by 5–7 days of lactation [8]. Placentae were collected at Days 14 and 20 of pregnancy. Experimental protocols were approved by the Boston University Institute Animal Care and Use Committee.

In Vitro Incubations

Adipose tissue and placentae were weighed, minced, and washed to remove residual extracellular fluid and blood cells with cold, serum-free Krebs buffer containing 4% (w/v) BSA and 0.1% (w/v) glucose. Adipose tissue from each animal was aliquoted into glass test tubes (~50 mg (wet wt)/tube in 1 ml of buffer). Adipose samples and placentae (1 minced placenta/tube in 1 ml of buffer) were incubated for as long as 240 min at 37°C in fresh buffer in a humidified O₂/CO₂ chamber with gentle shaking. At the end of the incubation periods, media were collected and stored at –20°C for subsequent RIA. The adipose tissue from each tube was collected and dried at 60°C for 3 days to obtain dry fat mass. Leptin concentrations in plasma and media were determined with a mouse leptin RIA kit (Linco, St. Louis, MO). The frozen media were first dried in a Speed-Vac (Savant Instruments, Inc., Farmingdale, NY) and then reconstituted to 10% of the original volume with assay buffer for RIA. This allowed duplicate determinations of leptin with values that fell on the linear portion of the leptin standard curve.

For long-term analysis of steroidal effects on leptin secretion and expression, fat tissue (~50 mg/animal) from normal, cycling mice (i.e., not controlled for stage of estrous cycle) was incubated in M199 with 4% BSA, L-glutamine, 25 µg/ml leupeptin, and penstrep. Corticosterone (Sigma Chemical Co., St. Louis, MO) and either estradiol-17β or estradiol benzoate (Sigma) were prepared in stock concentrations with ethanol and diluted in medium to reach the final concentrations used. Control tubes received a volume of diluted ethanol equivalent to the amount present in the highest steroid concentration (0.2% v/v). The tubes were incubated as described earlier, but for 24 h. At that time, the media (i.e., infranatant) were removed, dried, and reconstituted in RIA buffer for leptin RIA. Adipose tissue was collected from the tubes and dried in a convection oven to constant mass.

Implants

To test the effects of estradiol-17β and corticosterone on leptin secretion, mice were given s.c. implants containing either steroid on Day 3 of lactation. The implants were prepared as described previously by Widmaier and Campbell [24] to a filled length of 1 mm, allowed to equilibrate for 24 h in 20 ml of water at room temperature, and then

surgically implanted s.c. in the midscapular region. Animals were anesthetized with halothane during the procedure and immediately returned to their home cages with their pups after surgery. Control animals received empty implants. After 48 h, when plasma leptin levels are normally very low (see *Results*), animals were killed by exposure to carbon dioxide. Trunk blood was collected for hormone determinations, and adipose tissue was removed for immediate analysis of leptin secretion rates as described earlier.

Miscellaneous

Plasma corticosterone, progesterone, and estradiol were determined by RIA using commercially available kits (ICN, Costa Mesa, CA). For estradiol, plasma was first extracted with 10 volumes of heptane. Extraction efficiency was approximately 93%. For progesterone and leptin analysis in medium from adipose or placental tissue secretion experiments, the medium or Krebs buffer was dehydrated in a Speed-Vac, reconstituted to 10% of the original volume with RIA buffer, and then added to the RIA. Blank tubes containing medium or buffer (not exposed to incubated tissue) were similarly treated and subjected to RIA. No progesterone or leptin was detectable in the blanks. The volumes of plasma, concentrated media, or extract were used over ranges that diluted in parallel with the linear portion of the respective standard curve. Recovery of exogenous hormone added to the samples was 82%, 96%, 107%, and 109% for estradiol, progesterone, leptin, and corticosterone, respectively. Intra- and interassay coefficients of variation were, respectively, approximately 12% and 7% for estradiol, 9% and 21% for progesterone, 8% and 17% for leptin, and 15% and 10% for corticosterone.

Placentae pooled from three pregnant (g17) mice were cultured according to the method described by Thordarson et al. [25]. Isolated cells were separated by Percoll gradient centrifugation and cultured as monolayers for as long as 10 days. Media were collected every 24 h and centrifuged to remove floating debris. The supernatant was dried and reconstituted to 5% of the original volume with RIA buffer and then subjected to RIA. Blank tubes (i.e., media not exposed to cultured cells) were treated similarly, and those values, which were either negligible or undetectable, were subtracted in the RIA.

Data were analyzed by one- or two-way (no repeated measures) ANOVA followed by Student's *t*-test with Bonferroni correction for individual differences. Data were first subjected to Levene's test of homogeneity of variance and the Anderson-Darling test of normality before ANOVA. If these criteria were not met, data were ln-transformed before analyses. This transformation resulted in normality and homogeneity of variance for each instance in which it was used. Comparisons for two-way ANOVA were between group (e.g., pregnancy vs. nonpregnancy) and group × time.

To determine the half-maximally effective concentration of corticosterone on leptin secretion, data were subjected to best-fit nonlinear (unweighted) regression analysis using the method of least squares (PRISM software; GraphPad Software, Inc., San Diego, CA), with the constraint that the data conform to a sigmoidal dose-response curve with a single E_{max} and median effective concentration (EC_{50}). Data in Figure 3 were subjected to best-fit analysis by the method of least squares to generate the regression line and coefficient of determination (r^2).

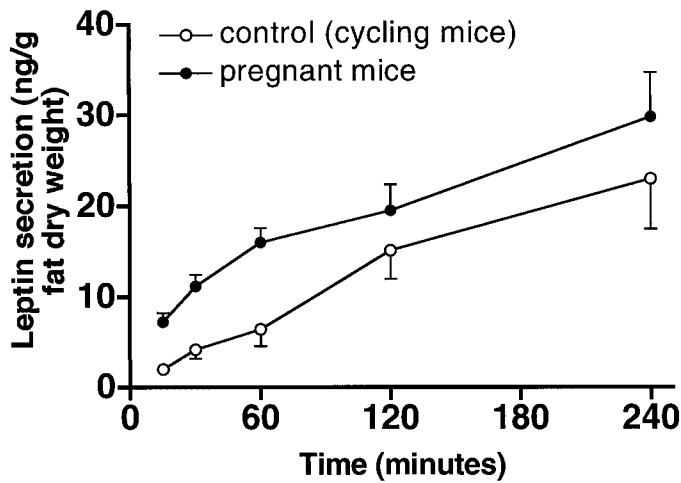


FIG. 1. Time course of leptin secretion in vitro from adipose tissue obtained from mice at Day 20 of gestation (closed circles, $n = 6$) or from nonpregnant female mice (open circles, $n = 6$). Values are means \pm SEM. The increase versus time was significant (ANOVA, $P < 0.0001$) in both groups. Leptin secretory rates from pregnant and nonpregnant mice were significantly different from each other by two-factor ANOVA. Data are normalized to gram (dry mass) of fat.

RESULTS

Immunoreactive leptin was secreted during a 240-min period from unstimulated maternal adipose tissue in vitro. An example of the pattern of secretion is shown in Figure 1 for tissue obtained on Day 20 of gestation. Leptin secretion from unstimulated adipose tissue of cycling, nonpregnant mice is also shown for comparison (Fig. 1). The two curves in Figure 1 significantly differed from each other by two-way ANOVA ($P < 0.001$), but no significant interaction (i.e., both increased with time with a similar pattern) was found. In a separate experiment, leptin secretion was determined in cycling mice at 3 h of incubation in vitro; the value for leptin secretion in that experiment was 18 ng/g dry fat (not shown).

To compare reproductive conditions, the total amount of leptin secreted during 240 min was compared between adipose tissue obtained from pregnant (Day 7, 14, or 20 of gestation) and lactating (Day 1, 2, or 5) mice. Plasma was also obtained at this time for leptin determination. The plasma leptin level was significantly lower at the end of the first week of pregnancy compared to that in nonpregnant, cycling mice (Fig. 2, top). By the middle and end of pregnancy, however, the plasma leptin level was significantly higher than that in nonpregnant animals (Fig. 2, top). The plasma leptin level remained relatively high for the first 2 days of lactation, but by Day 5 of lactation, it was similar to circulating levels observed at the onset of pregnancy (Fig. 2, top). A similar profile was observed with leptin secretion from adipose tissue in vitro (Fig. 2, bottom). Both plasma leptin level and rate of leptin accumulation in media from adipose tissue in vitro were significantly correlated in pregnant and lactating mice (Fig. 3).

In vivo exposure for 48 h to either estradiol or corticosterone significantly elevated the rate of leptin accumulation in the media as subsequently determined in vitro (Fig. 4). The effect of estradiol was significantly greater (two-way ANOVA) than that produced by corticosterone, and corticosterone, but not estradiol, implants significantly elevated circulating corticosterone levels after 48 h (Table 1). Estradiol, but not corticosterone implants, significantly el-

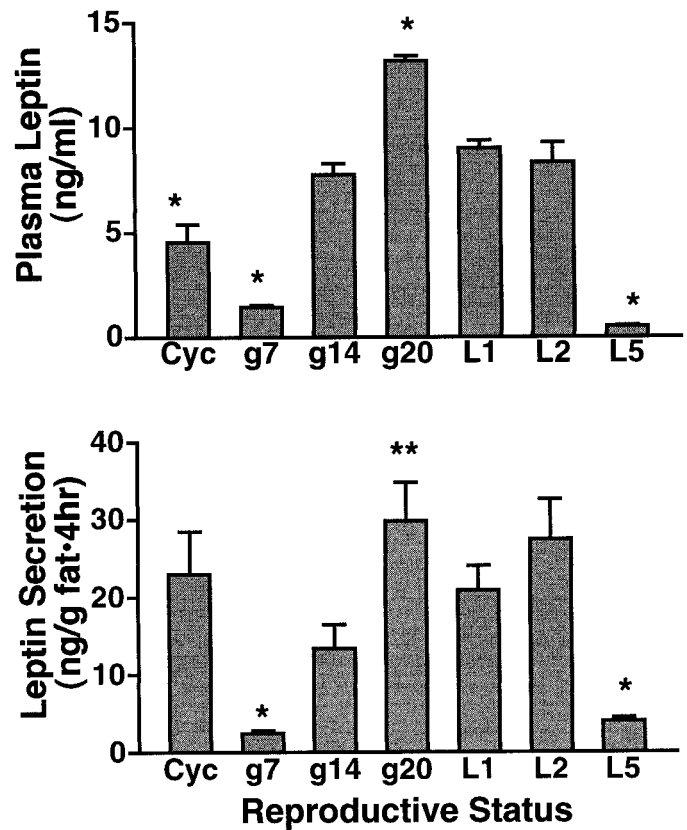


FIG. 2. Circulating (top) and secreted (bottom) levels of leptin (mean \pm SEM, $n = 4-7$) in cycling female mice (CYC); pregnant mice on Day 7, 14, and 20 of gestation (g); and on Day 1, 2, and 5 of lactation (L). Samples of blood and adipose tissue were obtained from the same animals. The values for cycling mice are from the 240-min time point in Figure 1 and are shown for comparison. *At least $P < 0.05$ versus all other groups, **at least $P < 0.05$ versus g7, g14, L1, L2, and L5.

evated circulating estradiol levels (Table 1). Surprisingly, though, neither hormone induced an increase in plasma leptin levels at the single time-point examined (Table 1).

When adipose tissue was removed from normal, cycling mice and directly exposed to steroid hormones in vitro, corticosterone significantly stimulated the leptin secretory rate (Fig. 5). The half-maximally effective concentration of

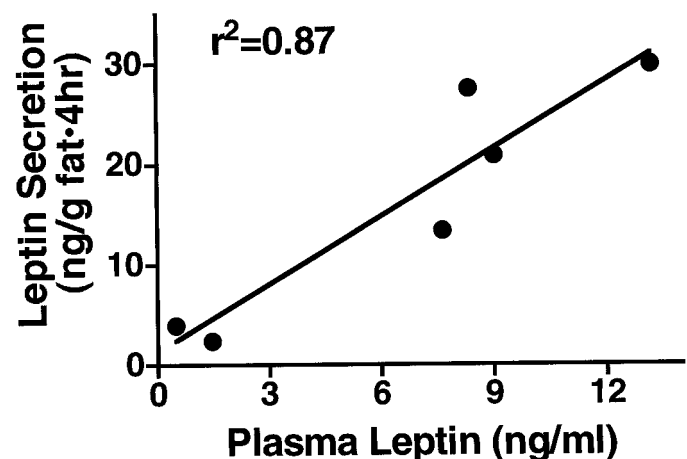


FIG. 3. Correlation between circulating leptin levels and 4-h secreted leptin levels from the adipose tissue shown in Figure 2. Each point is the mean value for one age group as shown in Figure 2.

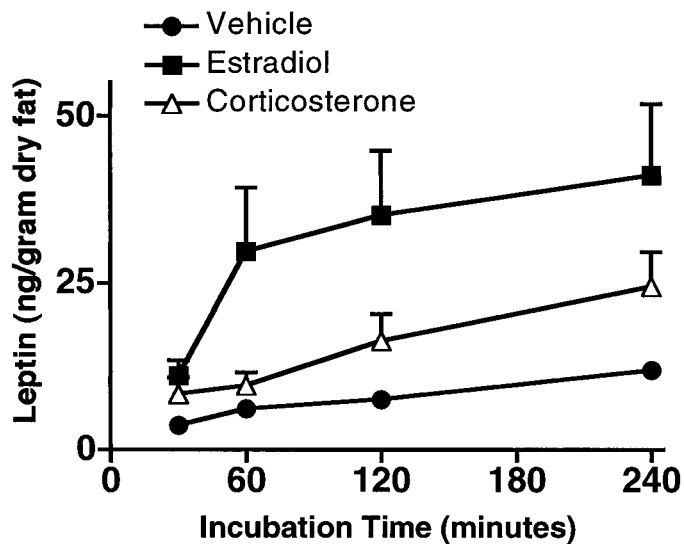


FIG. 4. Effect of s.c. implants of estradiol or corticosterone on subsequent leptin secretory rates in vitro. Steroid implants were present for 48 h, at which time the animals were sacrificed and the adipose tissue collected for in vitro analysis of leptin secretion. Both steroids significantly increased leptin secretory rates from adipose tissue in vitro compared with that in animals receiving empty capsules ($P < 0.0001$; two-factor ANOVA) and were also significantly different from each other ($P < 0.02$). Each point is the mean and SEM of 4–10 experiments. (Some error bars are obscured by the symbols.)

corticosterone, derived by nonlinear least squares analysis, was approximately 42 nM. Estradiol, either alone (Fig. 5) or in combination with a subeffective concentration of corticosterone (not shown), did not stimulate leptin secretion.

Minced placentae incubated in vitro did not significantly secrete immunoreactive leptin over 4 h, although the total amount of leptin appearing in the medium was increased between g14 and g20 (Fig. 6). These placentae, however, did secrete progesterone over the same time span ($r^2 = 0.96$ for progesterone content vs. time; data not shown). In addition, long-term monolayer cultures of mouse placental cells also failed to secrete leptin but did secrete approximately 5 ng of progesterone during the first 24 h of culture, which declined to low or nondetectable levels thereafter (not shown).

DISCUSSION

Recent reports have suggested that leptin may play roles in reproduction, including onset of puberty, regulation of fertility, and lactation [14]. During mid to late pregnancy, leptin levels increase in mice [5, 6], bats [7, 8], rats [3, 20], and women [9–12]. The source (or sources) of circulating leptin during pregnancy is currently unknown. However,

TABLE 1. Plasma hormone levels in mice implanted with empty or steroid-filled capsules.

| Hormone | Capsule type ^a | | |
|------------------------|---------------------------|-----------------------|--------------------------|
| | Empty (n = 10) | Estradiol (n = 7) | Corticosterone (n = 3–4) |
| Estradiol (pg/ml) | 156 ± 33 | 300 ± 40 ^b | 131 ± 33 |
| Corticosterone (ng/ml) | 194 ± 37 | 272 ± 25 | 444 ± 61 ^b |
| Leptin (ng/ml) | 11 ± 2 | 11 ± 2 | 12 ± 3 |

^a Values are mean ± SEM.

^b At least $P < 0.05$ versus respective empty capsule group.

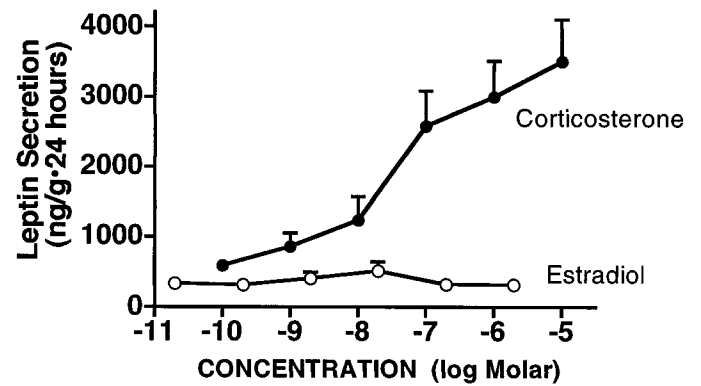


FIG. 5. Effect of corticosterone and estradiol on leptin secretion in vitro. Steroids were added for 24 h at the concentrations shown to minced adipose tissue from normal female mice. The medium was then collected and processed for leptin RIA. Each value is the mean and SEM of seven (corticosterone) or 15–16 (estradiol) animals. The effect of corticosterone was significant (ANOVA, $P < 0.0001$), with an estimated half-maximal concentration of 42 nM.

leptin mRNA levels increase in adipose tissue during pregnancy in rodents, with a time course roughly similar to that of the rise in circulating levels [3, 6], although this has not been universally observed [5, 20]. Whether increased mRNA expression is coupled with increased leptin secretory rates has not been directly tested by determining leptin secretion rates from adipose tissue in vitro. Here, we report that the in vitro rate of leptin secretion from adipose tissue of pregnant (and lactating) mice is highly correlated with circulating leptin levels from the same individuals. This strongly suggests that the putative up-regulation of adipose leptin mRNA during pregnancy [3, 6] is accompanied by increased constitutive leptin secretion, and that this continues for at least 2 days following parturition.

The factors responsible for up-regulating adipose leptin expression and secretion during pregnancy are unclear, but two possible candidates are estradiol and glucocorticoids. As with leptin, the circulating concentrations of these hormones increase during pregnancy. Both hormones also increase leptin gene expression in rodent [22, 23, 26–29] and

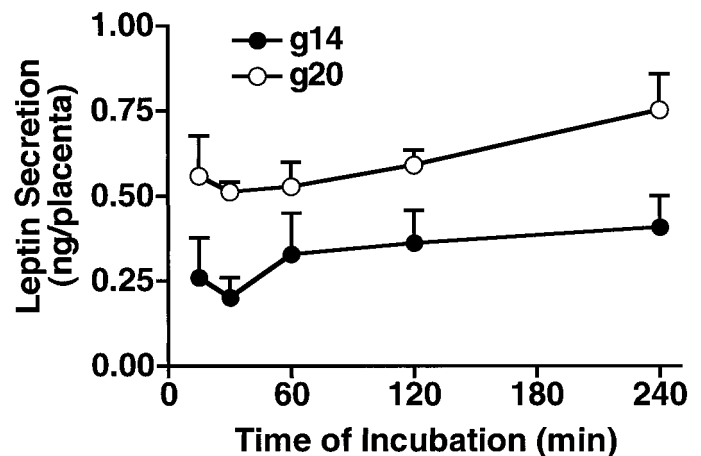


FIG. 6. Placental secretion of immunoreactive leptin in pregnant mice at Days 14 and 20 of gestation. Placentae were removed, minced, washed, and incubated for as long as 4 h. The two curves were significantly different ($P < 0.0001$) by two-way ANOVA. No significant interaction was found between the curves, however, and no significant effect of time was found. Each point is the mean and SEM of results from six animals.

human [28, 30, 31] adipose tissue. For example, adrenalectomy or ovariectomy decreases leptin mRNA levels in adipose tissue among rodents, and these effects are reversed by glucocorticoid [27] or estradiol [23, 28, 29, 32] replacement, respectively. In addition, changes in the circulating estradiol level during menstrual cycles or menopause positively correlate with the serum leptin levels in women [11, 28, 33, 34]. Other studies, however, have failed to demonstrate a correlation between circulating estradiol and leptin levels during the cycle in premenopausal women [35], in women taking birth control pills [36], or in cycling or ovariectomized rats [37]. Thus, the actions of estradiol and glucocorticoids on leptin gene expression may be subtle or even transient [27] enough that only rather drastic procedures (e.g., adrenalectomy) unmask the effects of each steroid in nonpregnant animals.

Here, we show that the functionally relevant end point of leptin gene expression, namely secretion of leptin, is stimulated by 2 days of *in vivo* exposure to estradiol or corticosterone. We chose to examine this in mice during the early stages of lactation (Day 5), because at that time, leptin secretion rates are significantly reduced below those of cycling or pregnant mice. The levels of corticosterone achieved by the implants were higher than those found in cycling mice but lower than those in pregnant mice in our experience (e.g., 900–1200 ng/ml between g14–g20). The levels of estradiol achieved by the implants were higher than those reported in the literature for pregnant mice [38], which may, however, reflect differences in assay procedures (e.g., extraction vs. nonextraction). By contrast, when corticosterone or estradiol were added directly to adipose tissue *in vitro*, only corticosterone stimulated leptin secretion. The half-maximally effective concentration of corticosterone (~42 nM) was within the range of circulating corticosterone levels during pregnancy.

No effect of estradiol was detected at any concentration, with or without concomitant additions of corticosterone, for 24 h. This result differs from that of another report demonstrating direct stimulatory effects of both dexamethasone and estradiol on leptin secretion from human adipose tissue [39]. However, the results from the latter study differed from ours in that pharmacological concentrations of estradiol were used and the steroid was present for as long as 96 h. Our results, therefore, suggest that short-term *in vivo* effects (i.e., implants) of estradiol may be mediated indirectly, possibly via stimulation of another leptin-inducing molecule, or be pharmacological. Although significantly different from each other, the plasma corticosterone levels after either estradiol or corticosterone implants were similar. Because estradiol raises plasma corticosterone levels in female rodents (probably through its action on corticosteroid-binding globulin), part of the *in vivo* action of estradiol on leptin secretion may be mediated through corticosterone.

Surprisingly, although the implants increased plasma estradiol and corticosterone levels, no change was observed in the plasma leptin level among the same animals in which the adipose leptin secretory rate was up-regulated *in vitro*. Because we only sampled blood at sacrifice, we do not know if the steroid treatments resulted in transient changes in leptin or changes that occurred at other times of the day. Integrated leptin levels over the entire 48-h period of steroid exposure may have revealed subtle differences that were missed by single samples. If, however, plasma leptin is, indeed, unaffected by short-term estradiol or glucocorticoid treatment in lactating mice, despite these steroids (directly or indirectly) up-regulating the leptin secretory rate,

then leptin clearance rates may be elevated by steroid hormones *in vivo*. We have no direct evidence as yet, however, to support this hypothesis.

Nonadipose sources of leptin also might account for the observed changes in circulating leptin during pregnancy. The placenta, for example, has been postulated as being a site of nonadipose leptin production. Human [10, 16–18] and baboon [40] placentae express leptin mRNA, but the situation is less clear in rodents [3–6, 20, 21]. Inconsistent with the hypothesis that placenta provides a major contribution to circulating leptin during pregnancy, however, is that plasma leptin levels do not return to baseline until several days after parturition and expulsion of the placenta [5, 8]. One possible explanation for this is that adipose tissue continues to secrete leptin at an accelerated rate for a brief time after birth, as demonstrated by the present study.

Leptin was not significantly secreted from placental tissue *in vitro* during a 4-h incubation period. In fact, leptin levels in media from isolated placentae barely changed over 4 h, suggesting that what was measured in the medium may have resulted from dissociation of blood-borne leptin from possible association sites in the placentae. The same tissue secreted progesterone, however, indicating that the placentae were viable. Moreover, long-term cultures of mouse placental cells, which would not be subject to any contamination with blood-borne leptin, also failed to secrete leptin, but they did secrete progesterone. These results strongly suggest that placenta does not contribute to hyperleptinemia of pregnancy in mice, but the possibility that circulating factors could induce placental leptin synthesis and secretion under the appropriate conditions should not be ruled out.

A reduction in leptin clearance rates secondary to changes in circulating leptin binding-proteins has been suggested to contribute to the progressive increase in plasma leptin levels during pregnancy in mice [5, 41]. This hypothesis is consistent with the present results, in which leptin secretory rates *in vitro* among cycling mice were 9.4-fold higher than those during early pregnancy (g7) despite plasma leptin levels that were only 3.2-fold higher. Only at the end of pregnancy do both secretory rates and plasma leptin levels exceed those of cycling, nonpregnant animals.

Thus, in early gestation, plasma leptin levels are low, which results, in part, from reduced leptin secretory rates by a currently unknown mechanism. This may permit the increased fuel deposition that occurs during this time, when fetal energy demands are low and the pregnant female must prepare for the increased metabolic demands of late pregnancy and lactation. As pregnancy proceeds, leptin secretory rates increase, and (possibly) leptin clearance rates decrease [5, 41], resulting in increased plasma leptin levels. That this triphasic change in secretory rates occurs from high (cycling) to low (early gestation) to high again (late gestation) implies that leptin is under tight regulatory control during these periods. The increased basal metabolic rate characteristic of mid to late gestation in pregnant mammals [12, 42] might result, in part, from hyperleptinemia at that time. During late pregnancy, mammals mobilize fat stores that were deposited during early pregnancy, when leptin secretory rates and plasma levels were still relatively low, to meet the increasing metabolic demands of the growing fetus. Concomitant with the increase in maternal metabolic rate is a decrease in feeding, partly because physical constraints begin to limit food consumption. These changes in metabolism and feeding are both consistent with an increase in the plasma leptin level.

After birth, leptin levels remain elevated for a short time.

The present results reveal that this is not simply a “wash-out” phenomenon, whereby high leptin concentrations are gradually cleared from the circulation. Instead, leptin secretory rates from adipose tissue remain high for at least 2 days after birth. Maternal leptin from lactating mothers has been demonstrated to appear in the circulation of suckling neonates [43]. Thus, the brief period of hyperleptinemia during early lactation may provide the suckling offspring with a source of leptin. Thereafter, maternal leptin levels decline as the leptin secretory rates decline, which likely is a mechanism whereby hyperphagia is stimulated during lactation.

The control of leptin during pregnancy appears to be taxa-dependent. For example, in two species of New World bats, the placenta secretes leptin *in vitro*, and its secretion is up-regulated during pregnancy [44]. Secretion of leptin from adipose tissue, however, changes little during pregnancy in bats. The human placenta expresses leptin mRNA and secretes leptin [45], but whether the rate of secretion increases during pregnancy remains unknown. Thus, mammals from three different orders (i.e., Primates, Rodentia, Chiroptera) develop hyperleptinemia during pregnancy, and they accomplish this by multiple mechanisms. The conservation of hyperleptinemia during pregnancy across mammalian orders implies a fundamental role of leptin in the maintenance of pregnancy and/or preparation for lactation.

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