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Dexamethasone Stimulates Leptin Release From Human Adipocytes: Unexpected Inhibition by Insulin

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Abstract In the present study we have examined the effect of dexamethasone on *ob* gene mRNA expression and leptin release from isolated human subcutaneous adipocytes. Dexamethasone stimulated leptin release from cultured adipocytes in a time- and dose-dependent manner. A two-fold increase in leptin release was detectable by 36 h of treatment with 10^{-7} M dexamethasone. Leptin release was preceded by a significant 83±30% increase in ob mRNA after 24 h exposure to the compound. Co-incubation of cells with dexamethasone (10^{-7} M) and insulin (10^{-7} or 10^{-9} M) completely blocked the dexamethasone-stimulated increase in *ob* mRNA and leptin release. These data demonstrate that insulin and glucocorticoids regulate leptin synthesis and release from human adipocytes in vitro. J. Cell. Biochem. 65:254–258. © 1997 Wiley-Liss, Inc.

Key words: ob gene; leptin; human adipocytes

The concentration of leptin in human serum has been demonstrated to be in proportion to the amount of adipose tissue mass in the body [Considine et al., 1996a]. However, serum leptin falls with fasting [Kolaczynski et al., 1996] and rises with massive overfeeding [Kolaczynski et al., 1996a] in the absence of changes in adipose tissue mass. In addition, serum leptin levels change with developmental stage in children independently of the amount of adipose tissue [Hassink et al., 1996]. These observations suggest that serum leptin levels are influenced by factors in addition to the size of the adipose tissue depot. Of particular interest is the potential regulatory role of metabolic hormones such as insulin, growth hormone, and cortisol.

We have previously reported a positive correlation of insulin with serum leptin in humans [Considine et al., 1996a]. Furthermore, chronic insulin treatment both in vivo and in vitro stimulates leptin synthesis and release [Kolaczynski et al., 1996b]. Although the effect of glucocorticoids has not yet been examined in humans, in rodents, administration of either hydrocortisone (100 µg/g body weight) or dexamethasone (3.7 µg/g body weight) produced a significant increase in epididymal fat *ob* mRNA expression in the absence of weight gain [De Vos et al., 1995]. The effect of dexamethasone in this study was detectable within 24 h after a single injection. Murakami et al. [1995] have observed a four- to eight-fold increase in *ob* mRNA following 10 h of dexamethasone treatment of isolated rat adipocytes. In the studies of Slieker et al. [1996], hydrocortisone and dexamethasone increased ob mRNA in cultured rat adipocytes which resulted in a three- to four-fold elevation in leptin secretion into the medium.

In the present study we demonstrate that dexamethasone stimulates leptin release from isolated human adipocytes in culture. Unexpectedly, co-incubation with insulin inhibits the dexamethasone-induced increase in leptin release. The effect of dexamethasone and insulin

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on leptin release appears to be mediated by changes in cellular *ob* mRNA content.

MATERIALS AND METHODS

Adipocytes were isolated by collagenase digestion of outpatient subcutaneous adipose tissue biopsies or surgical samples as previously described [Considine et al., 1996b]. Adipocytes (2 ml packed cells) were incubated in 10 ml of DMEM/F12 + 10% FBS in 125 ml sterile polycarbonate erlenmeyer flasks (Corning; Corning, NY) at 37°C and 95%O_2/5%CO_2. At the termination of an experiment the medium was removed and frozen for quantitation of leptin. Total RNA was obtained by the method of Chomczynski and Sacchi [Chomczynski and Sacchi, 1987].

Leptin was measured as previously described [Considine et al., 1996a] using a commercially available kit (Linco Research, St. Charles, MO). Samples (100 µl) were measured in duplicate. The culture medium containing 10% FBS contained no detectable leptin nor did it interfere with the detection of added standard leptin. The within assay variation was 8.3% and the between assay variation 6.2% at 4.9 ng/ml. Leptin release is expressed as the ng of leptin released by the ml of packed cells used in the experiment.

Ob gene mRNA expression was measured by reverse transcription polymerase chain (RT-PCR) reaction as previously described [Considine et al., 1995, 1996a]. All comparisons between samples were made on the linear portion of the amplification curve (between cycles 20–35) and no product was obtained in the absence of reverse transcriptase. The data are expressed as the ratio of *ob* cDNA to actin cDNA. There was no difference in the amount of actin cDNA among the samples studied.

RESULTS

Primary isolated human adipocytes were cultured in DMEM/F12 + 10% FBS and the release of leptin into the medium determined by radioimmunoassay. As shown in Figure 1, control cells released leptin into the culture medium in a linear fashion over the 48 h culture period. Exposure to 10^{-7}M dexamethasone resulted in a significant increase in leptin release which was detectable after 36 h. Exposure of cells to lower concentrations of dexamethasone (10^{-9} or 10^{-11} M) had no significant effect on leptin release (n = 3; data not shown).

Incubation of adipocytes with 10^{-7} M dexamethasone in the presence of 10^{-7} M insulin resulted in inhibition of the dexamethasone-stimulated increase in leptin release. As illustrated in Figure 2, insulin completely blocked the dexamethasone-stimulated increase in leptin release detected at 36 h of treatment and attenuated the dexamethasone effect by 74 ±

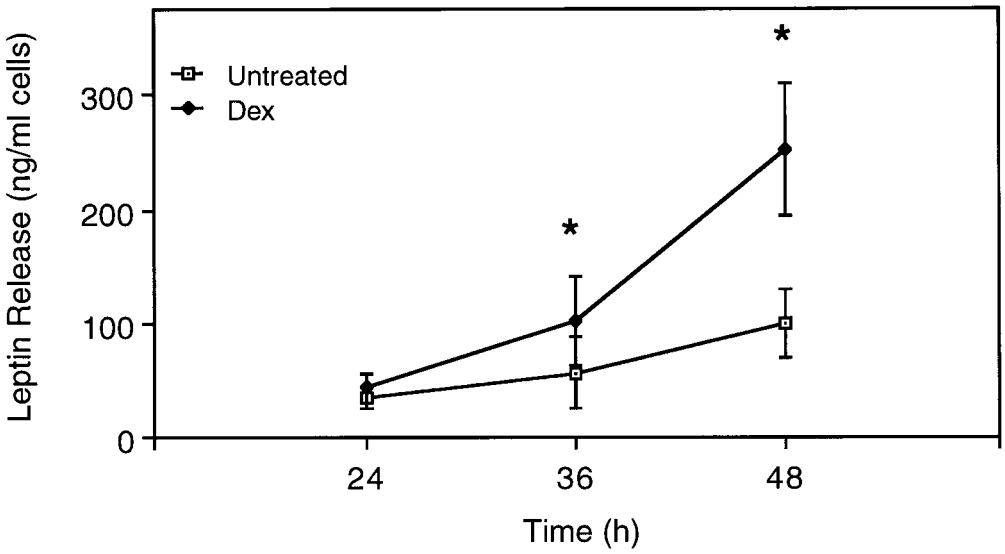

Fig. 1. Dexamethasone stimulates leptin release from human adipocytes in vitro. Subcutaneous adipocytes were cultured in DMEM/F12 + 10% FBS in the presence or absence of 10^{-7} M dexamethasone (Dex). Values represent the mean ± SEM for three separate experiments. *$P < 0.05$; Student's paired *t*-test comparison to control release.

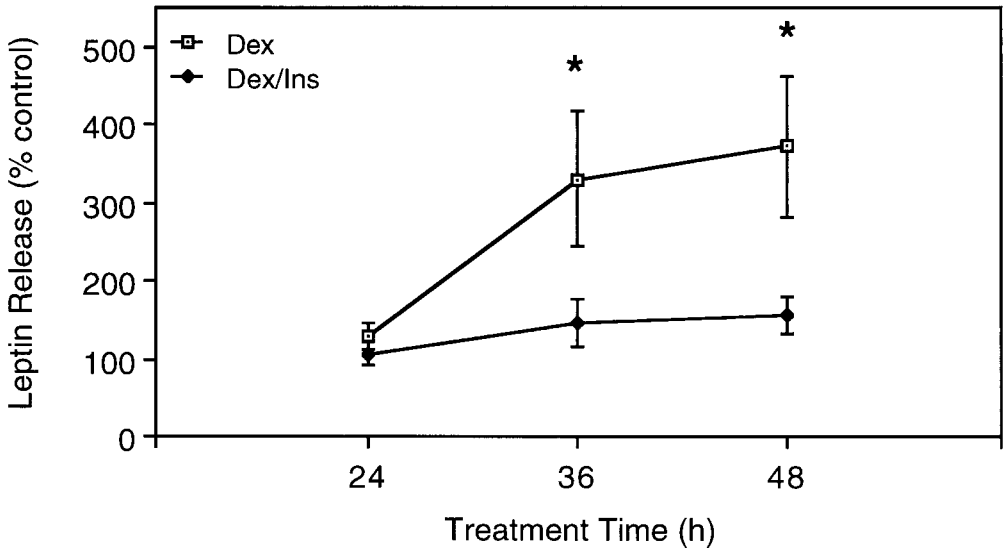

Fig. 2. Insulin inhibits the dexamethasone-stimulated increase in leptin release. Adipocytes were cultured in the presence of either 10^{-7} M dexamethasone (Dex) or 10^{-7} M dexamethasone + 10^{-7} M insulin (Dex/Ins). Values represent the mean ± SEM for three or more separate experiments. *$P < 0.05$; Student's paired *t*-test comparison to control leptin release.

13% following 48 h of exposure to the hormones.

The effects of dexamethasone and insulin on leptin release from human adipocytes appear to be mediated through changes in *ob* mRNA expression. A significant 83±30% increase in *ob* mRNA was detected following 24 h of treatment with dexamethasone (Fig. 3). The dexamethasone-induced increase in *ob* mRNA remained throughout the 48 h incubation period. The dexamethasone-induced increase in *ob* mRNA was completely blocked by co-incubation with 10^{-7} M insulin. The inhibition by insulin was dose-dependent as 10^{-9} M was as effective as 10^{-7} M. No inhibition of the dexamethasone-induced increase in *ob* gene mRNA was observed with 10^{-11} M insulin (Fig. 4). Exposure of adipocytes to 10^{-7} M insulin alone for 24 h resulted in a significant reduction in *ob* gene mRNA (55.1 ± 14.7 vs. 44.8 ± 12.9 relative units for the control and insulin-treated cells, respectively; $P < 0.025$ by Student's paired *t*-test comparison). Insulin alone had no effect on leptin release measured at 24 h.

DISCUSSION

We demonstrate in the present study that dexamethasone can stimulate leptin release from human adipocytes in a time- and dose-dependent manner. The dexamethasone-induced increase in leptin release appears to be mediated by an increase in *ob* gene mRNA content of the cells. We did not determine if dexamethasone increases *ob* mRNA transcription, message stability, or both. Surprisingly, co-incubation of adipocytes with insulin (10^{-7} and 10^{-9} M) blocked the dexamethasone-induced increase in *ob* mRNA and leptin release. Although treatment of cells with insulin (10^{-7}) alone resulted in an unexpected small but significant reduction in *ob* mRNA following 24 h of treatment, insulin-induced leptin release was not different from control at 24 h. We have previously reported that exposure of human adipocytes to insulin increases *ob* mRNA following 72 h of culture and leptin release by 96 h of culture [Kolaczynski et al., 1996b]. We have not observed a significant reduction in leptin release from adipocytes cultured in the presence of insulin at any time point studied. It is therefore possible that translation of *ob* mRNA is increased in the presence of insulin which offsets the reduction in mRNA content. Further experiments will be necessary to determine the contribution of transcription and translation to leptin production in isolated adipocytes.

A glucocorticoid response element has been identified in the human *ob* gene promoter region [Gong et al., 1996]. Furthermore, the ability of dexamethasone to increase *ob* mRNA and stimulate leptin release has been observed in rodent adipocytes [De Vos et al., 1995; Mu-

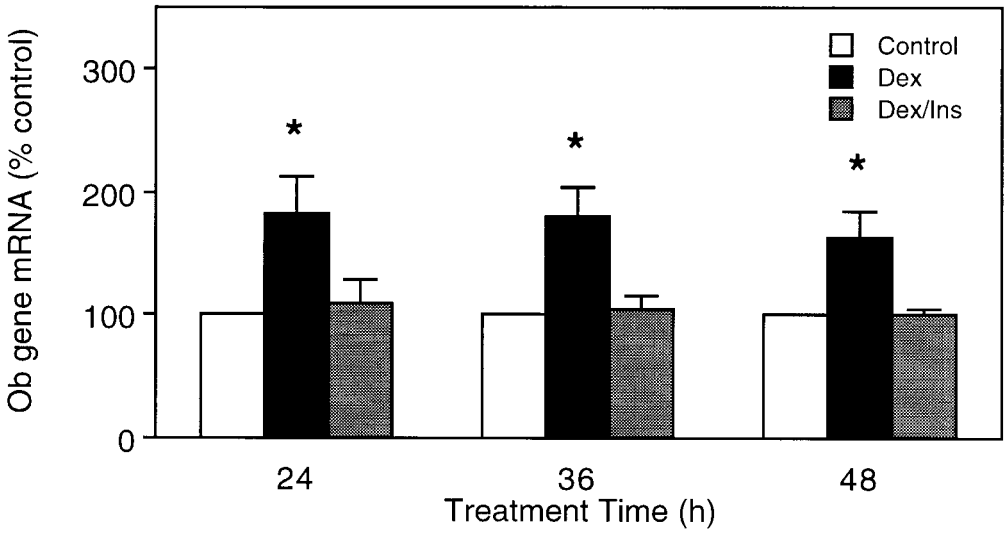

Fig. 3. Dexamethasone and insulin alter cellular *ob* mRNA content. Relative *ob* mRNA levels in adipocytes exposed to 10^{-7} M dexamethasone (Dex) $\pm 10^{-7}$ M insulin (Ins) were determined by RT-PCR. Values represent the mean ± SEM for three or more separate experiments. *$P < 0.05$; Student's paired *t*-test comparison to *ob* mRNA content in absence of treatment.

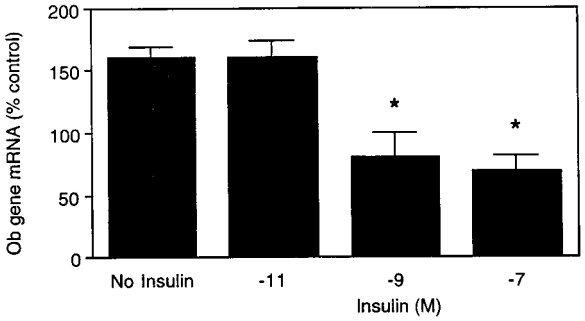

Fig. 4. Insulin inhibits the dexamethasone stimulated increase in *ob* gene expression in a dose-dependent manner. Cells were co-incubated with 10^{-7} M dexamethasone and decreasing concentrations of insulin. Values represent the mean ± SEM for three separate experiments. *$P < 0.05$; Student's paired *t*-test comparison to the dexamethasone-stimulated increase in gene expression.

rakami et al., 1995; Slieker et al., 1996]. In the one study to date on human cells, co-incubation with dexamethasone and insulin increases *ob* mRNA accumulation and leptin release [Wabitsch et al., 1996]. An important distinction between the study of Wabitsch et al. and the present report is the model used. Wabitsch and colleagues observed a synergistic effect of the hormones on human mammary preadipocytes that were differentiated to mature adipocytes in culture. Both insulin and dexamethasone were used to promote differentiation of these cells. It is therefore possible that insulin and dexamethasone may have different effects in these cells compared to that in the isolated mature adipocytes employed in the present study.

Insulin inhibition of a dexamethasone-induced increase in mRNA has been observed for other genes. Transcription of the peroxisomal acyl-CoA oxidase [Sorenson et al., 1993], insulin-like growth factor binding protein-1 [Suh et al., 1994; Goswami et al., 1994] and the phosphoenolpyruvate carboxykinase gene [Mitchell et al., 1994] is stimulated by dexamethasone and dominantly inhibited by insulin. Peroxisome proliferator-activated receptor (PPAR) has been suggested to be involved in the regulation of these genes [Steineger et al., 1994; Totonoz et al., 1995] and a similar mechanism to regulate *ob* gene expression may exist. The antidiabetic thiazolidinediones are high affinity ligands for the adipose tissue specific PPARγ [Lehmann et al., 1995], and it has been observed that *ob* mRNA is down regulated in rats after in vivo administration of the thiazolidinedione compound AD-5075 [Zhang et al., 1996]. Thiazolidinediones also reduce *ob* mRNA in differentiated 3T3-L1 cells [Kallen and Lazar, 1996] and isolated subcutaneous adipocytes [Nolan et al., 1996]. However, the mechanism by which PPARγ regulates *ob* gene promoter activity is complex since the usual PPARγ regulatory site has not yet been identified.

A final possible explanation for the observations in the present study is that the *ob* gene

promoter is responding to changes in lipid metabolism induced by dexamethasone and insulin and not to a direct effect of these hormones on the *ob* gene promoter. Further work will be necessary to determine the mechanism through which insulin and dexamethasone modulate cellular *ob* mRNA content and leptin release.

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