ORIGINAL ARTICLE

Dose-Dependent Cortisol-Induced Increases in Plasma Leptin Concentration in Healthy Humans

John W. Newcomer, MD; Gregg Selke, BA; Angela Kelly Melson, MA; John Gross, BA; George P. Vogler, PhD; Samuel Dagogo-Jack, MD

Background: Leptin is a hormone that regulates fat metabolism and appetite. The secretion of leptin is regulated by adiposity and, in the rodent, by factors such as insulin, β-adrenergic agonists, and glucocorticoids (GCs). Increased secretion of the endogenous human GC, cortisol, occurs during stress and in disorders such as major depression. Pharmacological GCs can robustly increase plasma leptin concentrations in humans, leading us to hypothesize that cortisol may serve as a physiological regulator of human leptin secretion.

Methods: A randomized double-blind placebo-controlled comparison of 2 fixed oral dosages of cortisol (40 mg/d and 160 mg/d), given for 4 days to matched groups of healthy subjects (n = 47). Low-dose treatment approximated GC output during mild stress, while high-dose treatment approximated GC output during maximal stress, spanning a range of GC secretion relevant to physiological stress.

Results: Cortisol produced dose-dependent and time-dependent increases in plasma leptin concentrations (time × treatment condition × body mass index; $F_{6,123} = 10.73$; $P < .001$). Initial treatment-induced increases in plasma leptin concentration returned toward baseline values during 4 treatment days, suggesting tolerance to this GC effect in these healthy subjects.

Conclusions: The results indicate an important role for GCs in the short-term regulation of human leptin secretion. Glucocorticoid-induced increases in leptin secretion suggest a mechanism that may contribute to anorexia and weight loss during stress and disease states such as major depression, if these conditions are associated with sustained increases in plasma leptin concentrations.

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From the Department of Psychiatry (Dr Newcomer, Messrs Selke and Gross, and Ms Melson) and Division of Endocrinology, Diabetes, and Metabolism (Dr Dagogo-Jack), Washington University School of Medicine, St Louis, Mo, and Department of Biobehavioral Health and Center for Developmental and Health Genetics, Pennsylvania State University, University Park, Pa (Dr Vogler).

GLUCOCORTICOIDS (GCs) are the product of a stress-responsive hypothalamic-pituitary-adrenal axis, which has been implicated[1,2] in the regulation of appetite and body weight. Research has been stimulated by identification of the mouse *obese* (*ob*) gene and its human homologue,[3] mutation of which in the mouse produces obesity. The *ob* gene product, leptin, can induce weight loss by decreasing food intake and increasing metabolic activity.[3-6] In humans, as in mice, leptin is secreted by adipocytes,[7-10] with plasma leptin levels highly correlated with total body adiposity.[8,11,12] Other factors regulating leptin levels in rodents include insulin, β-adrenergic agonists, and GCs,[13-17] with the latter increasing *ob* messenger RNA synthesis and leptin secretion and decreasing food intake.[15,16] In humans, the regulation[18-20] and metabolism[21] of leptin is less clear.

Investigators[22-26] have recently reported that pharmacological dexamethasone or methylprednisolone can increase plasma leptin concentrations in lean and obese human subjects across a wide age range, with only 1 study[27] failing to replicate this effect using methylprednisolone in lean men. These reports have generally indicated that women and individuals with higher, vs lower, body mass index (BMI; calculated as weight in kilograms divided by the square of height in meters: weight (kg)/[height (m)]^2) have more robust leptin increases in response to GC treatment. While these reports are clearly relevant to pharmacological treatment with dexamethasone or other GCs that have preferential affinity for type II GC receptors, it remains to be determined whether the endogenous human GC cortisol (CORT) plays an important role as a physiological regulator of human leptin secretion.

Partially addressing this question, investigators[28,29] have reported that leptin levels are increased in patients with Cushing disease even after controlling for increased BMI, although 1 study[30] found no decrease in leptin levels after correc-

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SUBJECTS AND METHODS

SUBJECTS

All subjects (N = 47) gave written informed consent for participation in a protocol approved by the Human Studies Committee at Washington University School of Medicine, St Louis, Mo. Subjects were recruited using local advertisement and screened for general medical health and psychiatric disorders using the Diagnostic Interview for Genetic Studies.[39] The inclusion criteria were subjects aged between 18 and 30 years and able to give informed consent. The exclusion criteria were as follows: (1) non–insulin-dependent or insulin-dependent diabetes mellitus; hypertension (treated or untreated); any major surgery in the preceding 6 months; any cardiac condition causing documented hemodynamic compromise; any respiratory condition causing documented or clinically recognized hypoxia; fever; dehydration; nausea; epilepsy; other endocrine disease; body weight less than 80% ideal body weight; any other medical condition requiring more than 7 days of hospitalization in the preceding 4 weeks; pregnancy or high-dose estrogen therapy; narcotic therapy; corticosteroid or spironolactone therapy; or psychotropic therapy; (2) any Axis I psychiatric disorder, including any substance use disorders; and (3) mental retardation as determined by *DSM-IV*[40] criteria. Clinical characteristics of the sample were as follows (±SD): mean age = 22.4 years (2.8 years); BMI = 23.19 kg/m^2 (3.95 kg/m^2); 23 men; 24 women. Thirteen women were taking oral birth control, and, of the remaining 11, 6 were in the luteal phase and 5 were in the follicular phase of their menstrual cycle at study baseline (as defined in the analysis section below). No significant differences in age ($F_{2,44} = 0.054$; $P = .9$), BMI ($F_{2,44} = 1.55$; $P = .2$), baseline plasma leptin levels ($F_{2,44} = 0.67$; $P = .5$), cortisol levels ($F_{2,44} = 1.59$; $P = .2$), insulin levels ($F_{2,44} = 0.009$; $P = .99$), or glucose levels ($F_{2,44} = 0.01$; $P = .99$) were detected across the high-dose CORT (7 men and 7 women), low-dose CORT (6 men and 8 women), or placebo-treated groups (10 men and 9 women). In addition, no significant main effect of sex or sex–by–treatment-condition interaction was detected for any of these variables.

PROCEDURE

The present study was a randomized double-blind placebo-controlled comparison of 2 fixed oral dosages of CORT (hydrocortisone), 160 mg/d and 40 mg/d, given for 4 days. All subjects received 1 of the 2 oral CORT doses or placebo using matched capsules given in divided doses (at 7 AM and 7 PM), using an approximately 3:2 dose ratio (high dose, 100 mg:60 mg; low dose, 25 mg:15 mg) to approximate circadian changes in endogenous CORT. Low-dose treatment approximated CORT output during mild physiological stress, while the high-dose treatment approximated CORT output during maximal physiological stress.[41,42] Plasma sampling and behavioral and adverse effect measurements were obtained at baseline (study day 0), after 1 day and 4 days of treatment (study days 1 and 4), and after a 6-day

tion of hypercortisolism. Ongoing studies[31] at this center suggest that plasma leptin concentrations in humans can be increased following continuous CORT infusions, with a leptin peak approximately 20 hours following the infusion onset. A recent study[32] of the pulsatility of leptin secretion indicated that leptin variability (rather than absolute level) was inversely related to adrenocorticotropic hormone and CORT variability. In addition, investigators[33] have recently identified a major quantitative trait locus on human chromosome 2 accounting for 47% of the variance in circulating leptin levels and containing several candidate genes, including the gene coding for pro-opiomelanocortin. The present study aimed to extend the previous literature by clarifying the role of stress-level CORT exposure in the short-term regulation of human leptin secretion.

Studies[34-36] during the past 30 years have documented complex changes in hypothalamic-pituitary-adrenal axis activity, including increased basal CORT secretion, in patients with major depressive disorder. Anorexia and weight loss are commonly found in clinically depressed patients. This is particularly a problem in the elderly, where depression is one of the most common causes of weight loss,[37] and both depression and weight loss are important predictors of poor functional outcome.[38] The reports reviewed above suggest that CORT may serve as a regulator of leptin secretion in humans, with potential relevance to physiological stress and pathophysiological mechanisms responsible for anorexia and weight loss in disorders such as major depression. The present study sought to test the hypothesis that short-term CORT exposure could increase plasma leptin concentrations using a randomized double-blind placebo-controlled parallel-group comparison of 2 fixed oral dosages of CORT spanning a range of cortisol secretion relevant to physiological stress. Each treatment was given for 4 days to well-matched groups of healthy subjects, with repeated plasma measurements at baseline, after 1 day, and again after 4 days of treatment.

RESULTS

Dose-dependent CORT-induced increases in plasma leptin concentrations were detected in both male and female subjects, with greater increases in plasma leptin in women vs men and in individuals with higher BMI. The initial analysis-of-variance model testing the effect of treatment condition and BMI on serial plasma leptin concentrations in the total subject sample detected an interaction between plasma sample time and treatment condition ($F_{6,123} = 8.18$; $P < .001$; G-G adjustment) and a 3-way interaction among sample time, treatment condition, and BMI ($F_{6,123} = 10.73$; $P < .001$; G-G adjustment). The strength of these results is explained in part by the observation that 100% of CORT-treated subjects demonstrated a numerical increase in leptin concentrations on day 1, compared with baseline. The

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washout period (study day 10). All assessments were performed at approximately 4 PM, with no food permitted after 1 PM. Previous investigations[43-46] have established that plasma leptin concentrations do not undergo significant diurnal variation in the absence of prolonged starvation or massive caloric loading.

PLASMA MEASURES

Plasma leptin concentration was determined by radioimmunoassay (Linco Research, St Louis, Mo), using purified recombinant human leptin as a standard and as a radiolabel and a rabbit polyclonal antibody to human leptin, with a second antibody separation method. The assay limits of detection and linearity were 0.5 ng/mL and 100 ng/mL in plasma,[47] with leptin values greater than 100 ng/mL assayed again on dilution. Intra-assay and interassay coefficients of variation were less than 7%, with no cross-reactivity with common peptide hormones. Plasma glucose was measured with a glucose oxidase method (Beckman Instruments, Fullerton, Calif). Plasma insulin[48] and total CORT[49] were measured by radioimmunoassay.

ANALYSIS

The comparability of the different treatment group characteristics (eg, age and BMI) was tested using analysis of variance. The main hypothesis concerning treatment effects on plasma leptin concentration was first tested using analysis of variance to evaluate leptin variance as a function of plasma sample time, treatment condition, and BMI. Body mass index was included even in the initial model based on previous work from this[22] and other laboratories indicating a critical effect of adiposity on leptin levels. The hypothesis was further tested using more inclusive analysis of variance and analysis of covariance models, that included subject sex and plasma covariate terms (CORT, glucose, and insulin). Significant effects were further evaluated using additional models and simple regressions within separate groups to better understand the contributions of individual continuous and factorial variables. To assess the validity of unadjusted F tests on the within-subject "time" factor and its interactions, the sphericity assumption for this data set was assessed using the test for sphericity applied to orthogonal components.[50] Because the test for sphericity was significant (Mauchly criterion, 0.288; $\sim \chi^2_5 = 53.2$; $P < .001$), it is appropriate to use P values for the univariate tests of hypotheses for within-subject effects that have the *df* adjusted. Two tests were applied that use adjusted P values, the Greenhouse-Geisser (G-G) and Huynh-Feldt adjustments. The former provided the most conservative estimate of significance in this analysis, and this test is presented below as appropriate. The effects of phase of menstrual cycle and oral birth control on plasma leptin concentrations were explored using unpaired t tests, comparing plasma leptin values across female subjects receiving oral birth control, female subjects in the luteal phase of their menstrual cycle (defined as day of cycle at baseline≤[cycle length in days/2]), and female subjects in the follicular phase of their menstrual cycle (defined as day of cycle>[cycle length in days/2]).

results can be further understood by examining male vs female subjects (**Figure 1**). A more inclusive model for predicting leptin levels detected a significant interaction among time, treatment condition, sex, plasma CORT concentrations, and BMI ($F_{18,98} = 3.47$; $P < .001$). This was explained by larger CORT-induced leptin increases in women (Figure 1), subjects receiving higher dose CORT treatment, and those with higher BMI (**Figure 2**). Importantly, CORT-induced increases in plasma leptin levels in these healthy subjects were detected after 1 treatment day, but were not sustained after 4 days of treatment, despite sustained CORT elevation.

Dividing subjects into obese (defined as men with BMI of >27.3 kg/m^2 and women with BMI of >27.8 kg/m^2)[51] vs lean individuals, regressions for plasma leptin levels vs CORT levels on day 1 were significant for low ($F_{1,40} = 5.78$; $P = .02$), but not high BMI, possibly because of the few obese subjects in our sample. Notably, the slopes of the regression lines for both lean and obese subjects were nearly identical (0.367 and 0.383, respectively), suggesting that data from additional obese subjects might fall along the same line. Higher mean plasma leptin levels in the male subjects receiving low-dose vs high-dose CORT treatment (Figure 1, bottom) were accounted for by a single obese male subject in the low-dose treatment group with higher baseline leptin and a robust CORT-induced leptin response. Exploring the effects of varying ovarian endocrine status in the female subjects using unpaired t tests, no differences in plasma leptin concentrations were detected among women in the luteal vs follicular phase of their menstrual cycle (as defined above) vs those receiving oral birth control at either baseline or at study day 1, consistent with most,[52,53] but not all,[54] previous reports.

As expected, a significant plasma sample time × treatment condition interaction was detected on circulating plasma CORT concentrations ($F_{6,132} = 9.54$; $P < .001$; G-G adjustment), with low-dose CORT treatment producing little change in plasma CORT concentrations measured at 4 PM compared with placebo, while the higher dose produced stable stress-physiological CORT concentrations at 4 PM throughout the 4 days of treatment (**Table**). A main effect of sex on plasma CORT levels ($F_{1,43} = 8.09$; $P = .007$) was explained by higher overall CORT levels in women vs men. In contrast to these differences in overall levels, treatment conditions produced a similar pattern of effects on plasma CORT levels across men and women (time × treatment condition × sex interaction: $F_{6,123} = 1.64$; $P = .17$; G-G adjustment). This result indicates that sex differences in leptin response are probably not explained by sex differences in the plasma CORT response to fixed CORT doses. A significant treatment effect on plasma glucose levels (time × treatment condition: $F_{6,132} = 5.19$; $P < .001$; G-G adjustment) was explained by a treatment-induced rise in plasma glucose during the high-dose CORT condition only (Table).

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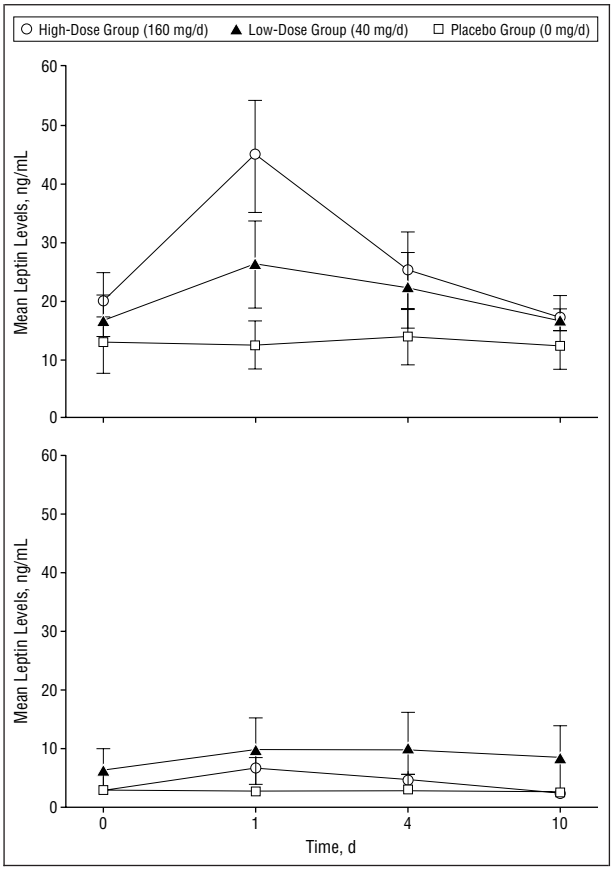

Figure 1. *Cortisol-induced increases in plasma leptin concentration in healthy female (top); (n = 24) and male (bottom); (n = 23) subjects (plasma sample time × treatment condition × sex: $F_{6,123}$ = 5.27; P<.001, Greenhouse-Geisser [G-G] adjustment). For women, both high-dose ($F_{3,18}$ = 11.97; P = .004; G-G adjustment) and low-dose ($F_{3,21}$ = 5.57; P = .02; G-G adjustment) cortisol conditions increased plasma leptin concentrations (P<.003 for contrast between day 0 and day 1 for both low dose and high dose and between day 1 and day 4 for high dose). For men, only the high-dose cortisol condition increased plasma leptin concentrations ($F_{3,18}$ = 7.12; P<.02; G-G adjustment; P<.002 for contrast between day 0 and day 1). Error bars indicate SE.*

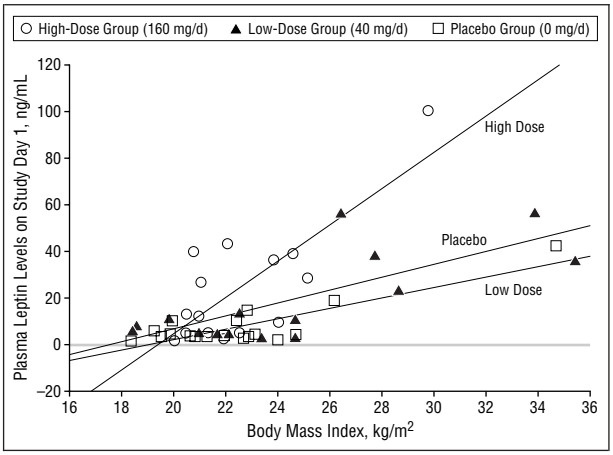

Figure 2. *Relationship between day-1 plasma leptin concentration and body mass index (BMI) for the entire treatment group (N = 47), as a function of treatment condition (treatment condition × BMI: $F_{2,42}$ = 6.90; P<.003). Regression for leptin (dependent) vs BMI was significant for the total sample ($F_{1,45}$ = 30.09; P<.001) with individual regressions (see displayed regression lines) within each treatment condition also significant at P<.003.*

No similar effects were detected on plasma insulin levels (time × treatment condition: $F_{6,132}$ = 0.88; P = .5; G-G adjustment) (Table).

COMMENT

The results of this study extend previous reports[22-26] that pharmacological doses of GCs can increase circulating leptin levels by establishing the significance of this effect for humans using CORT doses relevant to physiological stress. This study demonstrates dose-dependent and time-dependent CORT-induced increases in circulating leptin levels in young healthy humans, with greater dose-dependent CORT-induced increases in leptin levels in women and subjects with higher BMI. Body mass index remained a strong predictor of plasma leptin levels in this study as in our previous report,[22] interacting with CORT treatment such that greater CORT exposure in individuals with higher BMI produced the greatest increases in circulating leptin (Figure 2). Future investigations should include increased numbers of obese subjects to confirm the relationship of stimulated plasma CORT and leptin levels in obesity. Interestingly, plasma leptin levels escaped from the stimulating effects of sustained CORT exposure, suggesting tolerance to GC-induced stimulation or a homeostatic down-regulation of GC effects on leptin during the 4-day treatment interval in these healthy subjects.

In contrast, leptin levels remain increased over time in patients with Cushing disease even after controlling for increased BMI,[28,29] suggesting that some disease processes may interfere with the down-regulation of GC effects on leptin secretion. In addition, the failure of leptin levels to decrease after correction of hypercortisolism in patients with pituitary Cushing disease[30] suggests the possibility of long-term complex changes in mechanisms coupling GCs to leptin secretion. However, the interpretation of studies in patients with Cushing disease is complicated, with mechanisms coupling GCs to leptin secretion and other disease factors confounded. Future studies might address longer periods of cortisol treatment in healthy subjects and measure leptin activity in patients with major depression and other disease states associated with hypercortisolism and weight loss. Physiological CORT elevations in such diverse clinical states as depression, dementia, severe illness (eg, acquired immunodeficiency syndrome wasting syndrome), and stress might reduce appetite and weight, at least in part, through increases in circulating leptin levels. However, more basic studies confirming leptin effects on appetite and weight in humans are still needed.

Pharmacological doses of GCs have clinically recognized orexigenic effects, and GC-induced leptin secretion might be conceptualized as a counterregulatory mechanism limiting GC-related hyperphagia in all but pharmacological GC dosing conditions. Alternatively, some investigators[55] have proposed that chronic exposure to excess GCs might result in a down-regulation of leptin activity even to the point of leptin insensitivity and the development of obesity. The escape of plasma leptin levels from the stimulating effects of sustained CORT exposure in the healthy subjects we studied is consistent with this proposal and might be understood as an adap-

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Effect of Cortisol Treatment Conditions on Additional Plasma Measures*

	Treatment Day				
	0	1	4	10	*P*
Cortisol level, nmol/L†	. . .	. . .	. . .	. . .	<.001
Placebo group	301 ± 135	295 ± 160	293 ± 152	317 ± 215	.92
40-mg/d group	215 ± 105	232 ± 389	152 ± 163	248 ± 108	.50
160-mg/d group	279 ± 166	679 ± 400	662 ± 464	270 ± 132	<.001‡
Glucose level, mmol/L (mg/dL)	. . .	. . .	. . .	. . .	<.001
Placebo group	4.8 ± 0.9 (86.7 ±15.7)	4.6 ± 0.4 (82.5 ± 7.6)	4.7 ± 0.5 (84.9 ± 9.2)	4.8 ± 0.9 (86.1 ± 16.0)	.68
40-mg/d group	4.8 ± 0.4 (86.1 ± 7.8)	5.0 ± 0.5 (90.8 ± 9.3)	4.9 ± 0.8 (88.9 ± 14.5)	4.8 ± 0.4 (87.0 ± 6.5)	.47
160-mg/d group	4.8 ± 0.4 (86.5 ± 7.1)	5.9 ± 1.0 (106.8 ± 17.6)	5.5 ± 0.7 (98.7 ± 12.5)	4.8 ± 0.5 (87.2 ± 8.2)	<.001§
Insulin level, pmol/L‖	. . .	. . .	. . .	. . .	.51
Placebo group	83 ± 99	68 ± 53	89 ± 65	85 ± 62	.77
40-mg/d group	82 ± 79	80 ± 55	93 ± 70	98 ± 97	.83
160-mg/d group	80 ± 74	133 ± 52	152 ± 118	130 ± 133	.20

*Values for mean ± SD plasma measures across baseline (day 0), treatment (days 1-4), and treatment washout (days 5-10) in 47 healthy human subjects. Significance is given for treatment condition × time interactions for each plasma variable (see the "Results" section for F values) and for the main effect of time within each treatment condition. Ellipses indicate not applicable.

†To convert cortisol values to micrograms per deciliter, divide by 27.59.

‡$F_{3,39}$ = 12.98.

§$F_{3,39}$ = 12.72.

‖To convert insulin values to microunits per milliliter, divide by 6.0.

tive limitation to leptin-induced appetite reductions in healthy individuals when stress extends beyond 24 hours. However, whether this adaptation occurs in various disease states or can be sustained for longer periods in otherwise healthy subjects remains to be demonstrated. The escape of plasma leptin levels from GC stimulation in this study suggests that any role for leptin in weight and appetite reductions during disease-induced CORT elevations would require disruption of mechanisms responsible for tolerance to GC-induced leptin secretion.

This study was limited by the use of a single plasma sampling time, raising the concern that we might have missed an earlier CORT-induced leptin peak or that the peak may have shifted across study days so as to spuriously suggest tolerance. However, this is not likely to explain our results for several reasons. First, the time course for increases in leptin secretion in humans following a continuous CORT infusion indicates a leptin peak approximately 20 hours following the infusion onset,[31] consistent with previous reports of GC-induced increases in *ob* messenger RNA synthesis and not with GC effects on clearance alone. This time course suggests that our sampling time of 4 PM was well positioned to capture CORT-induced increases in plasma leptin concentration. Although the single plasma sampling time does not characterize the time course of leptin response to CORT, a focus of other research efforts, this was not the aim of this study. The present study sought to test the effect of CORT on leptin levels at a fixed representative point. In addition, there are currently no published data to suggest that the time course for CORT-induced leptin secretion shifts over time, and, assuming this response is related to a GC-induced increase in *ob* messenger RNA synthesis, we would not hypothesize such a shift.

This study was also limited by the use of 2 oral CORT dosing times to approximate physiological CORT secretion, allowing plasma CORT levels to decrease toward the end of each dosing interval. The relatively brief CORT plasma elimination half-life of 1.5 hours[56] may explain the observation that plasma CORT concentrations at 4 PM were no longer increased for the 40-mg/d group compared with the placebo group (Table). However, the biological (vs plasma elimination) half-life of CORT is 8 to 12 hours,[56] suggesting that CORT effects on leptin secretion could persist throughout the dosing interval. This distinction between the plasma elimination time and biological effects of CORT, as well as the continuous CORT infusion data from humans noted above, may explain why increases in plasma leptin concentrations were detected in female subjects even in the lower 40-mg/d CORT treatment group despite no increase in plasma CORT concentration at 4 PM.

While leptin effects on appetite, physical activity levels, and calorigenesis are well characterized in the rodent, the physiological significance of leptin activity in humans remains the subject of intensive ongoing investigation. In the future, it will be critical to define the short-term and extended physiological effects of increased plasma leptin concentrations in humans. In summary, the results of this study indicate an important physiological role for GCs in the short-term regulation of leptin secretion in humans. These GC-induced increases in leptin secretion suggest a mechanism that may contribute to anorexia and weight loss during stress and disease states such as major depression, if these conditions are associated with sustained increases in plasma leptin concentrations.

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Reprints: John W. Newcomer, MD, Department of Psychiatry, Washington University School of Medicine, 4940 Children's Pl, St Louis, MO 63110.

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