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Energy adaptation to glucocorticoid-induced hyperleptinemia in human beings

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Abstract

Recombinant leptin therapy potently decreases food intake and body weight in aleptinemic individuals with leptin gene mutations. However, it is unknown whether manipulation of endogenous leptin secretion alters ingestive behavior in otherwise healthy subjects. We therefore assessed energy consumption during administration of hydrocortisone (HC), a known leptin secretagogue. Six healthy adults were admitted overnight on 2 occasions and given HC (12.5 mg/h IV) or saline infusion for 24 hours (8:00 AM-8:00 AM) in a randomized crossover design. Total energy and macronutrient intake was calculated using a computerized nutrient analysis program. Blood sampling for measurement of leptin, cortisol, glucose, and insulin was performed at baseline and every 1 to 2 hours. A rise in plasma leptin level was noted after ~5 hours of HC infusion and was sustained throughout the study period. The total energy consumed was 3004 ± 231 kcal for saline and 2486 ± 214 kcal for HC ($P = .005$); breakfast energy values on day 1 were similar but energy values consumed at lunch, dinner, and day 2 breakfast were all significantly lower during induced hyperleptinemia. Analysis of macronutrients indicated significant decreases in carbohydrate and fat intake during glucocorticoid-induced hyperleptinemia as compared with placebo. These results indicate that stimulation of native leptin secretion decreases energy consumption, similar to the effect observed with recombinant leptin therapy. To our knowledge, this is the first report documenting anti-ingestive responses to a leptin secretagogue. Because chronic glucocorticoid therapy is fraught with adverse effects, we suggest that nonsteroidal or steroidomimetic leptin secretagogues may be good candidates for anorectic and antiobesity drug development.

1. Introduction

Administration of exogenous leptin results in profound weight loss in obese individuals with congenital leptin deficiency [1,2]. Similar but less dramatic benefits on weight reduction were observed after leptin augmentation in a cohort of 54 lean and 73 obese men and women with normal leptin genotypes (as indicated by baseline serum leptin levels >10 ng/mL) [3]. Furthermore, replacement doses of recombinant leptin normalized glucose and insulin levels in hyperglycemic, hyperinsulinemic *ob*/*ob* mice [4], leptin-deficient subjects with diabetes and insulin resistance [2], and hypoleptinemic patients with lipodystrophic diabetes [5]. These latter effects indicate that leptin has independent insulin-sensitizing properties. The weight loss after treatment with recombinant leptin results from inhibition of food intake and stimulation of physical activity [1,2] and is expressed as a selective loss of fat mass [1-3].

The effects of exogenous leptin therapy clearly indicate that the leptin-appetite-weight axis is active and can be manipulated in human beings, such that augmentation of the elevated leptin levels commonly found in obese subjects still exerts an anorexigenic effect [3]. However, it is unknown whether manipulation of endogenous native leptin levels (without recourse to injection of recombinant leptin) is a feasible strategy that can impact feeding behavior in human beings. Also unknown is whether any change in feeding behavior would occur in a physiologically meaningful time frame. Determination of the feasibility and biologic effects of endogenous leptin manipulation is of practical importance because recombinant leptin is not yet available for clinical use and is likely to be expensive when it becomes available. Our group [6-8] and others [9] have reported that plasma leptin levels are markedly increased

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(by ~100%) after single-dose or continuous administration of various glucocorticoids. In this report, we determined whether glucocorticoid-induced hyperleptinemia affects voluntary food intake in human beings.

2. Subjects and methods

Six healthy adults (3 women, 3 men) volunteered for the study protocol. The subjects had a mean (±SEM) age of 41 ± 1.7 years and a mean body mass index of 27.6 ± 0.9 kg/m^2. The subjects had no history of diabetes or current or previous use of glucocorticoids or other medications that alter appetite, body weight, or glucoregulatory physiology. No subject was enrolled in any active weight loss program. All subjects had normal fasting glucose levels and gave written informed consent before enrolling in this study, which was approved by the Washington University Human Studies Committee. Study subjects were admitted to the General Clinical Research Center after an overnight fast on 2 occasions separated by 2 weeks. On each occasion, the subjects received a continuous intravenous infusion of either hydrocortisone (HC, 12.5 mg/h) [7] or saline for 24 hours (8:00 AM-8:00 AM) in random fashion. Heart rate and blood pressure were monitored continuously during each 24-hour study period. Blood sampling for measurement of glucose, insulin, cortisol, and leptin was performed at baseline, hourly during the initial 6 hours, and every 2 hours for the remainder of the 24-hour period of infusion.

3. Computation of energy and macronutrient intake

By design, the study subjects had ad libitum access to meals and snacks during the study period. The subjects were given a printed menu from the hospital kitchen from which they were to order 3 meals and snacks on day 1 and breakfast on day 2. The study participants marked their food choices from the same menu during each entry. All meals and snacks were served on a tray and delivered by a research dietitian, who checked the food trays before and after meal/snack consumption. At the end of each mealtime, the uneaten items were recorded. The total energy and amounts of protein, fat, and carbohydrates consumed were estimated using the Computrition Recipe Nutrient Analysis program (RECANAL, Computrition, Inc, Chatsworth, Calif). The Exchange Lists for Meal Planning and published composition of food tables [10] were used for analyzing standard food portions such as bread/starch, fruit, milk, fat, and meat.

4. Biochemical analyses

Plasma leptin was measured with an in-house radioimmunoassay using a commercial kit (Linco Research, St. Louis, Mo). Samples were assayed in duplicate and the limits of detection and linearity for the leptin radioimmunoassay were 0.5 and 100 ng/mL, respectively; the intra-assay and interassay coefficients of variation were less than 7% [11]. Plasma insulin [12] and cortisol [13] levels were measured with radioimmunoassays. Plasma glucose was measured with a glucose oxidase method (Beckman Instruments, Fullerton, Calif).

5. Statistical analysis

Data are expressed as mean ± SEM. Serial plasma levels of leptin, insulin, cortisol, and glucose during HC and placebo treatments were analyzed using repeated-measures analysis of variance with Dunnett's test to confirm specific differences. Paired *t* tests were used to analyze differences in total energy intake, energy intake at specific meals, and macronutrient consumption during placebo and active infusions. Statistical analyses were run on an IBM StatView program (SAS Institute Inc, Cary, NC). A *P* value less than .05 was accepted as significant.

6. Results

6.1. Plasma glucose and insulin levels

Fig. 1 shows plasma glucose and insulin levels during HC and saline infusions. The mean plasma glucose levels at baseline were 99.5 ± 2.54 (saline) and 90.5 ± 1.80 mg/dL (HC, $P = .02$). Plasma glucose excursions were higher during the infusion of HC compared with those during infusion of saline. As expected, the postprandial increases in plasma glucose levels were exaggerated during glucocorticoid infusion. The differences between peak postprandial plasma glucose levels attained during HC vs saline infusion reached statistical significance after day 1 lunch ($P = .04$), day 1 dinner ($P = .01$), and day 2 breakfast ($P = .05$).

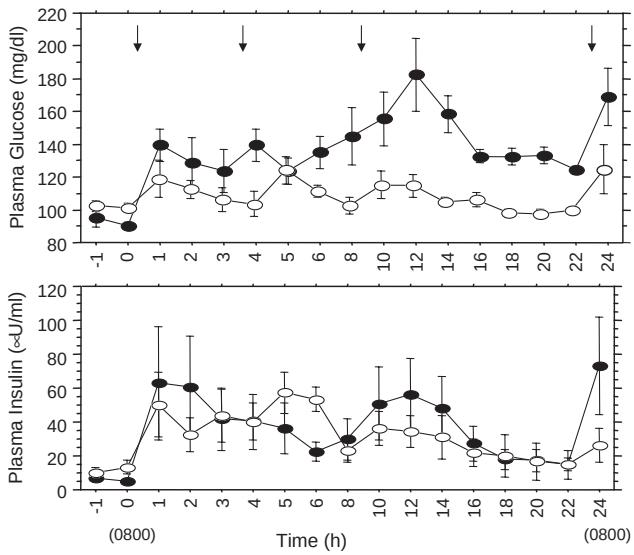

Fig. 1. Plasma glucose (upper panel) and insulin (lower panel) levels during infusion of HC (filled circles) and saline (open circles) in healthy subjects. Arrows indicate mealtimes.

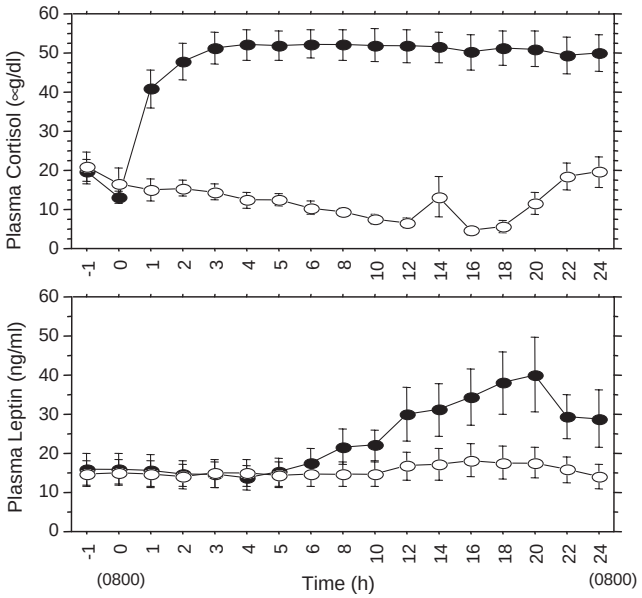

Fig. 2. Plasma cortisol (upper panel) and leptin (lower panel) levels during infusion of HC (filled circles) and saline (open circles) in healthy subjects.

Postprandial insulin levels also tended to be higher during HC infusion but the differences from placebo were not statistically significant (Fig. 1).

6.2. Plasma cortisol and leptin levels

Fig. 2 shows plasma cortisol and leptin levels during HC and saline infusions. The mean plasma cortisol levels at baseline were similar in the HC and saline arms (13.2 ± 1.59 vs 14.9 ± 2.12 μg/dL; $P > .5$). After 24 hours of saline infusion, plasma cortisol levels displayed the well-known diurnal decline and early-morning return to baseline on day 2. In contrast, HC infusion resulted in a sustained increase in plasma cortisol to ~50 μg/dL.

A measurable increase in plasma leptin was first noted after 5 hours of HC infusion and was sustained throughout the study period. Saline infusion did not alter plasma leptin levels. The basal and peak plasma leptin levels during saline infusion were 15.1 ± 3.3 and 18.2 ± 4.2 ng/mL, respectively ($P > .5$). The basal and peak plasma leptin levels during HC infusion were 16.0 ± 3.8 vs 42.1 ± 7.0 ng/mL ($P = .008$). A significant interaction was detected between change in plasma leptin level and treatment condition ($P = .0001$); in contrast, the change in plasma glucose or insulin levels did not predict leptin levels.

6.3. Energy consumption

As shown in Fig. 3, the total energy values from 8:00 AM on day 1 to 8:00 AMon day 2 were 3004 ± 231 (saline) and 2486 ± 214 kcal (HC, $P = .005$). Energy consumption at breakfast on day 1 was not significantly different during saline and HC infusions (581 ± 97.9 vs 579 ± 78.7 kcal; $P > .1$). However, compared with saline infusion, HC infusion was associated with a significant decrease in

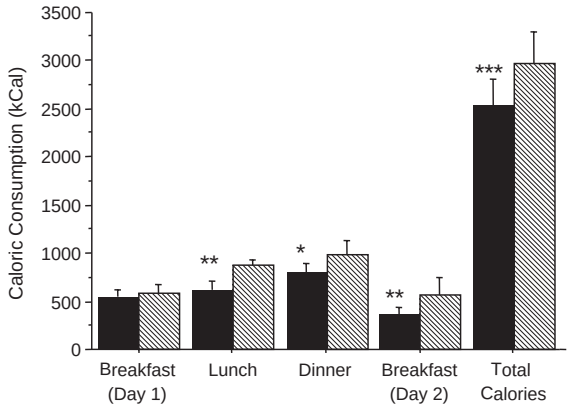

Fig. 3. Energy consumption during infusion of HC (filled bars) and saline (hatched bars) in healthy subjects. Asterisk represents $P = .04$; double asterisk, $P = .02$; triple asterisk, $P = .005$. To convert kilocalories to kilojoules, multiply by 0.239.

energy consumption during lunch (645 ± 63.4 vs. 916 ± 65.8 kcal; $P = .005$) and dinner (814 ± 64.9 vs 981 ± 96.3 kcal; $P = .04$). Energy intake at breakfast on day 2 was also significantly lower during glucocorticoid-induced hyperleptinemia compared with that during saline infusion (378 ± 72.3 vs 537 ± 99.8 kcal; $P = .02$).

6.4. Macronutrient consumption

Analysis of macronutrient consumption showed significant reduction in the intake of fats and carbohydrates during glucocorticoid-induced hyperleptinemia; protein energy consumption was unchanged (Fig. 4). Specifically, the amount of fats consumed during the 24-hour study period was 119 ± 15.1 g during saline infusion and 88.5 ± 14.9 g during HC infusion ($P = .004$). Carbohydrate consumption was 372 ± 36.0 (saline) and 311 ± 31.4 g (HC, $P = .01$); protein intake was 111 ± 12.5 (saline) and 115 ± 15.8 g (HC, $P > .1$).

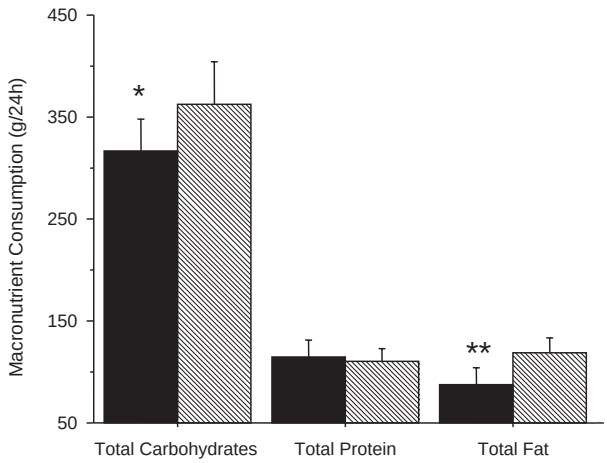

Fig. 4. Macronutrient consumption during infusion of HC (filled bars) and saline (hatched bars) in healthy subjects. Asterisk represents $P = .01$; double asterisk, $P = .004$.

7. Discussion

Glucocorticoids stimulate appetite [14], increase body weight, and induce insulin resistance [15]. The orexigenic effect of glucocorticoids is mediated, at least in part, by augmentation of the hypothalamic feeding signal neuropeptide Y (NPY) [16,17], an effect that is inhibited by leptin [18] and insulin [17]. The metabolic effects of leptin result from coordinate activation of anorexigenic pathways and inhibition of orexigenic pathways mediated by leptin-responsive neurons in the hypothalamus [19,20]. Because leptin has anorexigenic and insulin-sensitizing properties [1-5], we hypothesized [6] that glucocorticoid-induced hyperleptinemia is a physiological (counterregulatory) mechanism designed to limit the hyperphagia, weight gain, and insulin resistance associated with glucocorticoid excess.

If the foregoing notion is accurate, induction of endogenous hyperleptinemia by glucocorticoid treatment should result in a measurable decrease in energy intake after an initial increase in food intake owing to the orexigenic effect of glucocorticoids. Based on this premise, we had expected that any anorexigenic effect of glucocorticoid-induced hyperleptinemia would be preceded by evidence of increased food intake. Our data did not confirm the expected initial increase in energy intake after glucocorticoid infusion, as indicated by the similar or lower energy intake data at breakfast and lunch on day 1 of HC infusion compared with saline treatment. However, our data showed a potent inhibitory effect of endogenous hyperleptinemia on feeding behavior. This effect coincided with the onset of hyperleptinemia after HC treatment, which indicates that prior activation of NPY and central orexigenic stimuli is not required for glucocorticoid-induced hyperleptinemia to suppress food intake. Indeed, the latter conclusion is consistent with demonstration from in vitro studies that glucocorticoids can directly stimulate leptin mRNA synthesis in cultured adipocytes [21].

The interaction between glucocorticoids and leptin thus can be conceptualized as having a direct short-loop and an indirect long-loop component. The short loop involves direct activation of glucocorticoid receptors [22] in adipocytes, which results in stimulation of leptin synthesis [21] after a lag time of ~5 hours, consistent with a transcriptional mechanism. No prior activation of orexigenic signals appears to be required for activation of this pathway. On the other hand, the putative long loop operates indirectly by requiring that glucocorticoids first stimulate the expression of NPY in hypothalamic neurons, thereby increasing appetite and food intake, before "counterregulatory" secretion of leptin by the adipocytes. We [23] and others [24] have reported that the plasma leptin response to glucocorticoids is abolished by fasting. The latter observation indicates that, although antecedent hyperphagia was not observed in the present study, food ingestion is nonetheless permissive of glucocorticoid-induced hyperleptinemia.

The clinical significance of our finding—that endogenous hyperleptinemia induced by glucocorticoid suppressed food intake—needs to be interpreted carefully. Experiments of nature such as Cushing's syndrome, in which chronic hypercortisolemia results in chronic hyperleptinemia [25], are associated with obesity rather than anorexia and weight loss, as would have been predicted from our model. A similar experience applies to patients treated chronically with pharmacological doses of glucocorticoids. In contrast, patients with Addison's disease do not develop obesity during lifelong replacement therapy with physiological doses of glucocorticoids. Thus, the physiological effects of glucocorticoids (in orchestrating leptin responses that regulate ingestive behavior) may be quite different from the pharmacological effects of chronic steroid therapy or hypersecretion. Chronic exposure to pharmacological doses of glucocorticoids overrides the anorexigenic effects of the resultant hyperleptinemia, presumably, by increasing the expression of hypothalamic orexigenic neuropeptides [16,20,26] through activation of central glucocorticoid receptors [27].

In conclusion, our current and previous [6-8,23] data show that physiological doses of glucocorticoids stimulate leptin secretion in vivo. In the present report, we show for the first time that augmentation of native leptin secretion regulates food intake in healthy subjects. Our findings are relevant to the quest for antiobesity therapies that act via the leptin pathway. Because chronic use of glucocorticoids is fraught with obvious problems, glucocorticoids are not a practical choice for therapeutic manipulation of endogenous leptin levels. On the other hand, the discovery of nonsteroidal or steroidomimetic leptin secretagogues that have neutral side effects would be a major advancement in future drug development for treatment of obesity.

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References

[1] Farooqi IS, Matarese G, Lord GM, et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest 2002;110:1093-103.

[2] Licinio J, Caglayan S, Ozata M, et al. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A 2004;101:4531-6.

[3] Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose escalation trial. JAMA 1999;282:1568-75.
[4] Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the obese gene product on body weight regulation in *ob*/*ob* mice. Science 1995;269:540-3.
[5] Oral EA, Simha V, Ruiz E, et al. Leptin-replacement therapy for lipodystrophy. N Engl J Med 2002;346:570-8.
[6] Dagogo-Jack S, Selke G, Melson AK, et al. Robust leptin secretory responses to dexamethasone in obese subjects. J Clin Endocrinol Metab 1997;82:3230-3.
[7] Askari H, Liu J, Dagogo-Jack S. Hormonal regulation of human leptin in vivo: effects of hydrocortisone and insulin. Int J Obes 2000;24: 1254-9.
[8] Newcomer JW, Selke G, Melson AK, et al. Dose-dependent cortisol-induced increases in plasma leptin concentration in healthy humans. Arch Gen Psychiatry 1998;55:995-1000.
[9] Larsson H, Ahren B. Short-term dexamethasone treatment increases plasma leptin independently of changes in insulin sensitivity in healthy women. J Clin Endocrinol Metab 1996;81:4428-32.
[10] Pennington JAT. Bowes and Church's food values of portions commonly used. 15th ed. Philadelphia (Pa): Lippincott; 1989.
[11] Ma Z, Gingerich RL, Santiago JV, et al. Radioimmunoassay of leptin in human plasma. Clin Chem 1996;42:942-6.
[12] Kuzuya H, Blix P, Horwitz D, et al. Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 1977;26: 22-9.
[13] Farmer R, Pierce C. Plasma cortisol determination. Radioimmunoassay and competitive binding compared. Clin Chem 1974;20: 411-4.
[14] Dallman MF, Strack AM, Akana SF, et al. Feast and famine: a critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrinol 1993;14:303-47.
[15] McMahon M, Geriche J, Rizza R. Effect of glucocorticoids on carbohydrate metabolism. Diabetes Metab Rev 1988;4:17-30.
[16] Zarjevski N, Cusin I, Vettor A, et al. Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology 1993;133:1753-8.
[17] Wilding JPH, Gilbey SG, Lambert PD, et al. Increases in neuropeptide Y content and gene expression in the hypothalamus of rats treated with dexamethasone are prevented by insulin. Neuroendocrinology 1993;57:581-7.
[18] Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, et al. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 1995;377:530-2.
[19] List JF, Habener JF. Defective melanocortin 4 receptors in hyperphagia and morbid obesity. N Engl J Med 2003;348:1160-3.
[20] Schwartz MW, Woods SC, Porte D, et al. Central nervous system control of food intake. Nature 2000;404:661-71.
[21] Papaspyrou-Rao S, Schneider SH, Petersen RN, Fried SK. Dexamethasone increases leptin expression in humans in vivo. J Clin Endocrinol Metab 1997;82:1635-7.
[22] Feldman D, Loose D. Glucocorticoid receptors in adipose tissue. Endocrinology 1977;100:398-405.
[23] Umamaheswaran I, Askari H, Tykodi G, et al. Plasma leptin responsiveness to glucocorticoid occurs at physiological doses, and is abolished by fasting. Obes Res 2003;11:232-7.
[24] Laferrere B, Fried SK, Hough K, et al. Synergistic effects of feeding and dexamethasone on serum leptin levels. J Clin Endocrinol Metab 1998;83:3742-5.
[25] Masuzaki H, Ogawa Y, Hosoda K, et al. Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome. J Clin Endocrinol Metab 1997;82:2542-7.
[26] Kalra SP, Dube MG, Pu S, et al. Interacting appetite-regulating pathways in the hypothalmic regulation of body weight. Endocr Rev 1999;20:68-100.
[27] Spencer RL, Young EA, Choo PH, et al. Adrenal steroid type I and type II receptor binding: estimates of in vivo receptor number, occupancy, and activation with varying levels of steroid. Brain Res 1990;514:37-48.