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ORIGINAL ARTICLE

Administration of human leptin differentially affects parameters of cortisol secretion in socially housed female rhesus monkeys

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Abstract Chronic exposure to psychosocial stress may lead to a dysregulation of the limbic–hypothalamic–pituitary–adrenal axis that results in a number of adverse health outcomes. The fat-derived hormone leptin has been indicated as a potential key component to maintaining homeostasis by enhancing glucocorticoid negative feedback. Using an established model of nonhuman primate social stress, notably social subordination, this study examined the effects of continuous leptin administration on cortisol secretion in female rhesus monkeys. The 20 subjects were maintained in stable five-member social groups with established dominance hierarchies. All females were ovariectomized but received estradiol throughout the study to maintain serum concentrations at early follicular phase levels. Three parameters of cortisol secretion were examined in dominant and subordinate females during control and leptin-treatment conditions: diurnal cortisol secretion; response to a dexamethasone suppression test; and response to a brief separation from their social group. We hypothesized that leptin supplementation would attenuate the hypercortisolemia characteristic of subordinate females. During baseline conditions, subordinate female rhesus monkeys had significantly lower levels of serum leptin compared with more dominant monkeys and were less sensitive to glucocorticoid negative feedback. Exogenous administration of leptin improved glucocorticoid negative feedback in subordinate females and decreased morning cortisol in all animals. However, there were no status differences in response to a social separation test and diurnal rhythm in cortisol during baseline conditions. However, leptin administration did not attenuate the increase in cortisol in response to a social separation. The data presented in this study demonstrate that leptin can attenuate several parameters of cortisol secretion in female rhesus monkeys and thus may play a role in the response of the adrenal glands to socio-environmental stimuli.

Keywords Social subordination · Psychosocial stress · Cortisol · Leptin

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Introduction

Stress is an adaptive response to a physiological or psychological challenge to an organism's ability to maintain homeostasis. This is a beneficial reaction to an acute stressor that enables an individual to mobilize energy and respond to a physical or emotional challenge. Exposure to a chronic stressor, on the other hand, can be a detriment to both physical and behavioral health, as these conditions increase the risk for a wide range of adverse health outcomes, including accelerated progression of coronary artery disease [1, 2]; affective disorders [3, 4], eating disorders and metabolic dysregulation [5–8], addiction [9]; and reproductive compromise [10]. In addition, chronic activation of the limbic–hypothalamic–pituitary–adrenal (LHPA) axis also adversely affects immune function [11], increasing vulnerability to opportunistic disease [12, 13]. These adverse health outcomes are secondary to the dysregulation of the LHPA axis that occurs during exposure to chronic stressors [14, 15].

While continued exposure to socio-environmental stressors may result in the dysregulation of the LHPA axis [16–19], the consequences of chronic stress may itself

impair homeostatic mechanisms that regulate the stress axis. For example, the effect of chronic stressors on appetite and metabolism are complex and may depend on the availability of specific macronutrients [20]. However, a typical outcome of chronic stress exposure, particularly in animal models, is reduced food intake and a hypometabolic condition [21, 22], resulting in reduced leptin synthesis and secretion from adipocytes [23–25]. This reduction in leptin may, indeed, exacerbate LHPA dysregulation and thus perseverates any adverse health effects, as leptin can act centrally and peripherally at the level of the pituitary and adrenal to attenuate the activation of the LHPA axis [26, 27]. In addition, rodent models indicate that leptin reduces LHPA activation to a metabolic [28] and physical stressor [29–31] and reduces basal ACTH release and adrenal glucocorticoid release in non-stressed animals [32]. Leptin infusion also enhances glucocorticoid negative feedback and reduces glucocorticoid response to a novel situation in female rhesus monkeys [33]. Importantly, leptin deficient, ob/ob mice have elevated glucocorticoids [34] and leptin replacement normalizes basal LHPA levels in ob/ob mice [35, 36]. These data would suggest leptin is an important signal that can modulate the activity of the LHPA axis in response to socio-environmental stressors.

In contrast to physical stressors, social subordination is considered a chronic psychosocial stressor [37]. Social subordination in the macaque female is also an ethologically valid psychosocial stressor. When housed socially, female macaque groups, regardless of size, are organized by a linear dominance hierarchy that functions to maintain group stability [38, 39]. Lower ranking animals receive proportionately more aggression from higher-ranking group mates and these subordinates terminate these interactions by emitting submissive behaviors. Indeed, submissive behavior directed at other conspecifics is the defining feature of social subordination in macaque societies, with the lowest ranking animal submitting to all and the most dominant animal submitting to none [38–41]. Thus, control over an individual's social and physical environment increases with higher dominance status [42]. Given the recurrent exposure to harassment from more dominant females, subordinate females have larger adrenal glands [43] and show a greater cortisol response to social challenges [44]. In addition, pharmacological tests using a dexamethasone suppression [25, 45–49] or ACTH challenge [49] show subordinate females are hypercortisolemic. The use of social subordination in macaques is a well-established model to study the adverse effects of psychosocial stress on cardiovascular disease [50], addictive behavior [51], central monoamine changes [49, 52, 53], reproductive dysfunction [54], immune compromise [47, 55], appetite [45], and an increase in anxiety-like or displacement behaviors [45, 56] known to be stress dependent [57]. Previous studies have also shown that subordinate adult female rhesus monkeys are hypoleptinemic compared to more socially dominant cage mates [25].

Using socially housed adult female rhesus monkeys, this study tested the hypothesis that leptin supplementation would attenuate the hypercortisolemia characteristic of subordinate females. Three parameters of cortisol secretion were examined in dominant and subordinate females during control and leptin-treatment conditions: diurnal cortisol secretion; response to a dexamethasone suppression test; and response to a brief separation from their social group.

Results

Subject characteristics and treatment efficacy

As illustrated in Fig. 1, the amount of aggression received and the amount of submissive behavior emitted was linearly related to dominance rank. Using previously established conventions [43], females ranked 1 or 2 in their group were classified as dominant and those ranked 3, 4, or 5 were considered subordinate. These social dominance ranks were unchanged throughout the course of the study. Leptin administration had no effect on behavior (data not shown).

Estradiol treatments produced similar serum levels of the hormone in dominant and subordinate females (Table 1). Anthropometric and basal hormone data are shown in Table 1. Overall, dominant animals weighed more ($F_{1,17} = 9.03$, $P < 0.02$) and had a higher body mass index (BMI, $F_{1,17} = 7.08$, $P < 0.02$) but not sagittal abdominal diameter (SAD) ($F_{1,17} = 1.80$, $P > 0.05$) than subordinate subjects. Treatment with E2 resulted in a significant decrease in weight ($F_{1,17} = 70.58$, $P < 0.01$) and BMI ($F_{1,17} = 70.26$, $P = 0.02$) but not SAD ($F_{1,17} = 2.55$, $P > 0.05$) for both

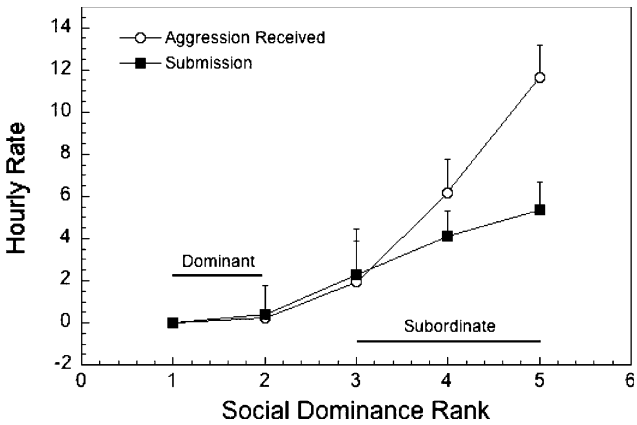

Fig. 1 Mean ± SEM hourly rates of aggressive behavior received from other cage mates and submissive behavior directed at others as a function of female rank. Using established conventions [43], females ranked 1 and 2 are considered dominant and females rank 3–5 considered subordinate

Table 1 Mean ± SEM anthropometric measures and serum estradiol concentrations for dominant and subordinate females during both phases of the study

Measure	Dominant	Subordinate	Statistics
Body weight (kg)			
E2 start	7.70 ± 0.22	6.67 ± 0.19	Status: $P < .01$
E2 end	7.46 ± 0.22	6.55 ± 0.19	Status × RX: NS
E2 + leptin start	7.50 ± 0.28	6.49 ± 0.24	Rx × time: NS
E2 + leptin end	7.38 ± 0.29	6.39 ± 0.25	
BMI			
E2 start	13.2 ± 0.4	11.4 ± 0.3	Status: $P = .04$
E2 end	12.5 ± 0.5	10.9 ± 0.4	Rx: NS
E2 + leptin start	12.9 ± 0.7	11.5 ± 0.6	Rx × time: $P < .01$
E2 + leptin end	12.5 ± 0.7	11.3 ± 0.6	
SAD (cm)			
E2 start	9.00 ± 0.28	8.28 ± 0.24	Status: NS
E2 end	8.46 ± 0.23	8.11 ± 0.19	Status × Rx: NS
E2 + leptin start	8.81 ± 0.31	8.31 ± 0.26	Rx × time: $P < .01$
E2 + leptin end	9.94 ± 0.36	8.33 ± 0.1	
E2 (pg/ml)			
E2	27.9 ± 4.6	30.4 ± 3.8	NS
E2 + leptin	27.6 ± 8.3	32.7 ± 8.6	

Significant main effects of status and treatment (Rx) and the interaction of these effects are illustrated in the "statistics" column and explained more fully in the text

dominant and subordinate monkeys but leptin did not exacerbate these effects ($P > 0.05$). Between status groups, leptin treatment had no significant consequence on weight ($F_{1,17} = 0.25$, $P > 0.05$), BMI ($F_{1,17} = 0.31$, $P > 0.05$), or SAD ($F_{1,17} = 0.56$, $P > .05$).

As seen in Fig. 2, serum leptin levels were significantly lower in subordinate monkeys throughout the control condition ($F_{3,54} = 2.75$, $P = 0.05$). These status differences were maintained through day 5 of the leptin-treatment phase. However, by day 14 of treatment, leptin concentrations were significantly elevated to a similar concentration in both dominant and subordinate females ($F_{3,54} = 69.23$, $P < 0.01$).

Diurnal hormone levels

Secretion of cortisol was examined at 0900, 1200, and 1800 hours for both the control and leptin infusion phases (Fig. 3). All females show the expected diurnal rhythm in serum cortisol ($F_{2,36} = 26.45$, $P = 0.01$), with highest levels in the morning compared to noon and early evening values ($P < 0.01$). However, there was no significant difference in cortisol levels for dominant versus subordinate animals across the three time points ($F_{1,18} = 1.23$, $P = 0.28$) nor was there a significant interaction between status and time of day ($F_{1,18} = 0.88$, $P = 0.42$). There was

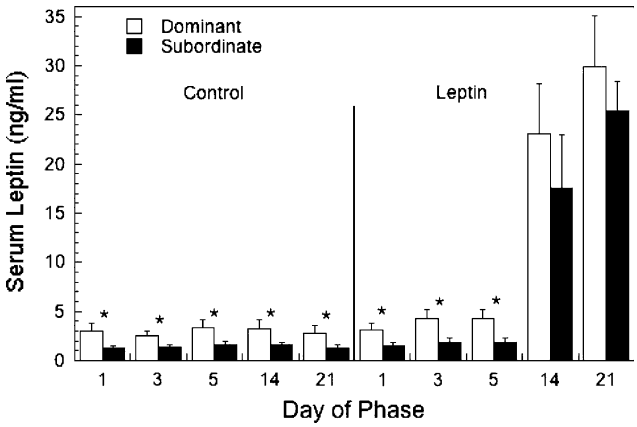

Fig. 2 Mean ± SEM serum levels of leptin during both control and leptin treatment conditions for dominant and subordinate females. Leptin levels were significantly lower in subordinates compared with dominant females throughout the control and through day 5 of leptin treatment (indicated by an *asterisk*, $P < 0.05$)

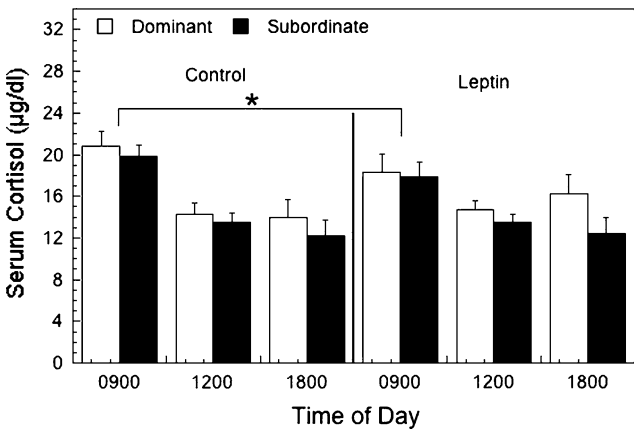

Fig. 3 Mean ± SEM diurnal serum concentration of cortisol obtained at three time points throughout the day in dominant and subordinate females. Leptin significantly attenuated morning serum cortisol concentrations (indicated by an *asterisk*, $P < 0.05$). There were no differences in diurnal cortisol between dominant and subordinate females ($P > 0.05$)

no main effect of leptin administration ($F_{1,18} = 0.12$, $P = 0.73$) or a leptin by status interaction ($F_{1,18} = 0.19$, $P = 0.67$). However, there was a significant leptin by time interaction ($F_{2,36} = 5.98$, $P = .01$). Post-hoc tests revealed that leptin infusion significant reduced morning levels of cortisol ($P < 0.05$) but not noon or early evening levels (Fig. 3).

Glucocorticoid negative feedback

A dexamethasone suppression test was used to determine glucocorticoid negative feedback and LHPA responsivity (Fig. 4). Dexamethasone effectively suppressed serum cortisol at 15, 18, and 19 hours post-administration in all

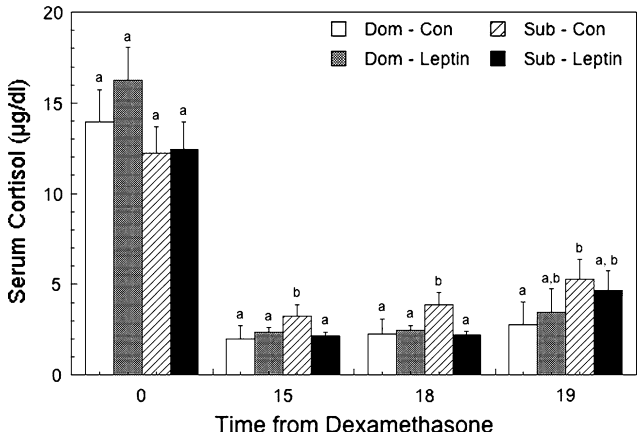

Fig. 4 Mean ± SEM serum concentrations of cortisol obtained prior to and following an injection with dexamethasone at 1800 hours in dominant and subordinate females. Subordinate females had significantly higher concentrations of cortisol following dexamethasone compared to dominant females during the control condition (indicated by *different letters*, $P < 0.05$). However, leptin improved sensitivity to dexamethasone in subordinate females, as post-injection values were similar to those observed in adults ($P > 0.05$). Leptin infusion did affect the response to dexamethasone in dominant animals ($P > 0.05$)

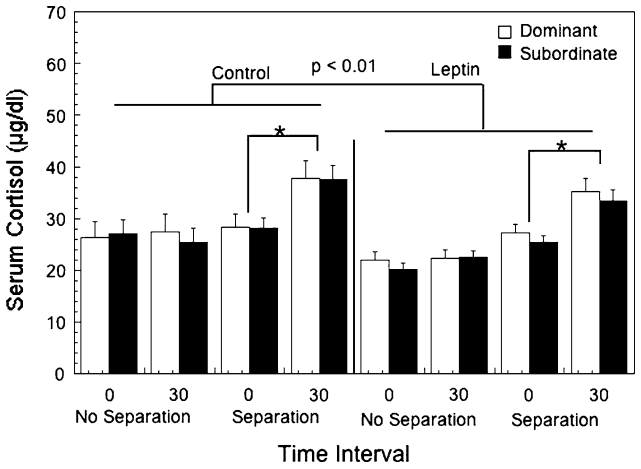

Fig. 5 Mean ± SEM serum concentration of cortisol obtained at time 0 and 30 min later following a control (no separation) and social separation test for dominant and subordinate females. Social separation significantly increased serum cortisol during both conditions (indicated by an *asterisk*, $P < 0.05$). Leptin significantly attenuated serum cortisol at all time point during the non-separation control and separation test ($P < 0.05$)

females ($F_{3,54} = 98.27$, $P = 0.03$). Although the main effect of status was not significant ($F_{1,18} = 1.23$, $P = 0.23$), there was a significant status by time interaction ($F_{3,54} = 3.36$, $P = 0.03$), as subordinate females were less sensitive to dexamethasone suppression than were dominant females during the control condition. The administration of leptin did not affect all females similarly ($F_{1,18} = 0.07$, $P = 0.90$). Rather, it improved glucocorticoid negative feedback in subordinates ($F_{1,18} = 4.32$, $P = .05$), eliminating the social status differences observed during the control phase. As illustrated in Fig. 4, subordinate females had significantly higher serum cortisol levels at +15 and +18 hours following dexamethasone during the control phase compared to the leptin-treatment phase and to dominant females during both phases. At the +19 hours time point, subordinates had significantly higher serum cortisol compared with dominant females during the control phase, whereas values during the leptin-treatment phase were intermediate.

Response to a novel environment

The activation of the LHPA axis following social separation and placement in a novel environment was examined (Fig. 5). While cortisol did not increase at the 30-min sample on non-separation days, serum levels were significantly elevated after the 30-min separation in both dominant and subordinate females ($F_{1,18} = 13.93$, $P < .01$). Indeed, the response in cortisol did not differ between dominant and subordinate animals ($F_{1,18} = 0.03$, $P = .86$) and the magnitude of the increase in serum cortisol following social separation did not differ between the Control (9.42 ± 1.61 µg/dl) and leptin-treatment phases (8.00 ± 1.35 µg/dl; $P > 0.05$). Leptin administration significantly attenuated overall serum cortisol levels ($F_{1,18} = 6.47$, $P = 0.02$) and this attenuation was not different between dominant and subordinate animals ($F_{1,18} = 0.09$, $P = 0.76$).

Discussion

This study shows that dominant females are heavier and have higher estimates of body fat compared with subordinate females. During baseline conditions, subordinate female rhesus monkeys have significantly lower levels of serum leptin compared with more dominant monkeys and are less sensitive to glucocorticoid negative feedback. However, there were no status differences in response to a social separation test and diurnal rhythm in cortisol. Importantly, exogenous administration of leptin improved glucocorticoid negative feedback in subordinate females and decreased morning cortisol in all animals but did not attenuate the increase in cortisol to a social separation.

The results of this study are consistent with previous data examining the relation between leptin and LHPA responsivity. Leptin can act centrally and peripherally at the level of the pituitary and adrenal to attenuate the activation of the LHPA axis [26, 27]. In addition, rodent models indicate that leptin reduces LHPA activation to a metabolic [28] and physical stressor [29–31] and reduces

basal ACTH release and adrenal glucocorticoid release in non-stressed animals [32]. Leptin deficient, ob/ob mice have elevated glucocorticoids [34] and leptin replacement normalizes basal LHPA levels in these animals [35, 36]. Leptin infusion also enhances glucocorticoid negative feedback and reduces glucocorticoid response to a novel situation in female rhesus monkeys [33].

The present results show that the administration of leptin normalizes glucocorticoid negative feedback in subordinate monkeys. Glucocorticoid negative feedback is characteristically reduced in subordinate female macaques relative to dominant females [25, 45–49]. During leptin administration, the response to dexamethasone was increased in subordinates compared to that observed in dominant monkeys. Dominant animals were unaffected by leptin, suggesting that these animals were maximally responsive to the dose of dexamethasone used in the study. Furthermore, disrupted diurnal cortisol rhythms are associated with adverse early experience [58, 59] or mood disorders [60]. Consequently, we predicted that subordinate females may show an altered diurnal pattern of cortisol and that leptin would normalize this rhythm. However, there were no social status differences in diurnal cortisol but leptin administration did attenuate the morning elevation in circulating levels in both dominant and subordinate females. These data contrast previous observations that leptin infusion did not affect the diurnal rhythm in cortisol [33]. An explanation is not readily apparent but females in the previous study were late adolescent female rhesus monkeys, whereas those used in this study were prime aged adults. Because morning cortisol may show an age-dependent increase in females as they mature [61], it is possible that the regulation by leptin of this parameter of cortisol secretion is developmentally regulated as well. This can only be fully addressed by prospective studies.

Finally, because previous exposure to stressors enhances the response to a novel stressor [62], we hypothesized that subordinate females may respond with a greater increase in cortisol to a brief social separation test, a paradigm that elicits a robust LHPA and behavioral response in monkeys [63, 64]. However, serum cortisol increased similarly to the separation in dominant and subordinate females. Furthermore, leptin did not attenuate the increase in serum cortisol in response to the social separation. However, leptin administration did significantly lower serum cortisol concentrations at all time points throughout the non-separation condition on day 7 and the social separation condition on day 10. Because these tests occurred in the morning, this effect of leptin is consistent with the decrease in morning serum cortisol observed diurnal assessments on day 15. It is clear that the social separation is a potent activator of adrenal cortisol secretion in all females. Thus, it would seem the leptin is capable of attenuating some aspects of cortisol secretion but not under particularly challenging situations.

Our study cannot determine how leptin influenced peak diurnal levels of cortisol or improves glucocorticoid negative feedback in subordinates. Previous studies show that leptin reduces the release of cortisol from adrenocortical cells in vitro [65, 66] by down-regulating P450 side chain cleavage expression [67]. However, because leptin administration did not uniformly decrease cortisol concentrations in all samples, it seems unlikely that this effect on glucocorticoid biosynthesis accounts for the effects observed in this study. While leptin receptors are expressed in the pituitary, no co-localization is found in corticotropes [68]. However, the possibility exists that leptin inhibits ACTH from vesicles in the corticotropes. Leptin receptors are located in many parts of the CNS [69], underscoring the nature of its pleiotropic effects [70–74] including neuroprotection [75] and anxiolytic action [76]. Although the anorexic effects of leptin may, in part, be mediated by an increased expression of CRF in the PVN [77], leptin increases GR expression in areas known to mediate glucocorticoid negative feedback [78–80], including the PVN and hippocampus [81]. Thus, leptin may act to improve glucocorticoid negative feedback by increasing central GR expression. Because peak circadian levels of glucocorticoids are influenced by the expression of central GRs [82], any effects of leptin on central GR expression may also modulate these peak concentrations.

The results of this study suggest that leptin can attenuate several parameters of adrenal cortisol secretion in female rhesus monkeys. Hypometabolic conditions and reduced leptin secretion are often associated with elevated cortisol release [83]; however, it is difficult to dissociate stress induced from the metabolic-induced increases in glucocorticoids under these situations. In contrast, greater fat mass is often associated with activation of the LHPA in animal models [84] and humans [85]. Since obesity is thought to be associated with leptin resistance [86], it is possible that the greater activation of the LHPA axis in these individuals is due to reduced leptin efficacy. Indeed, diet-induced type II diabetes, resulting in leptin resistance, is associated with changes in both GR and MR expression in the limbic system [87]. Importantly, the notion that increases in serum leptin attenuate morning cortisol and improves glucocorticoid negative feedback in subordinates has implications for understanding the shift in diet preferences for calorically dense foods during periods of stress [88, 89]. Consumption of these diets does attenuate the response to stressors in rodent models [90–92], suggesting that a metabolic signal acts centrally to diminish LHPA activation. Although circulating levels of leptin did not explain differences in LHPA responses in rats fed normal chow versus a high-caloric diet [92], the results of this study suggest that the role of leptin in attenuating stress responsivity warrants further investigation.

Methods

Subjects were 20 adult female rhesus monkeys (*Macaca mulatta*) housed in four groups of five animals each at the YNPRC Field Station. Groups were housed in runs that had an indoor and an outdoor area. Females ranged in age from 8 to 12 years and all had been ovariectomized 5 to 7 months prior to the study [25, 93]. Animals were fed commercial monkey chow (Purina Mills, St. Louis, MO, USA) ad libitum twice daily and seasonal fresh fruit and vegetables daily. The Emory University Institutional Animal Care and Use Committee, in accordance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act, approved all procedures.

Dominance positions within each group were determined empirically based on the outcome of dyadic interactions in which a female clearly emitted a submissive response to another animal [38]. Using previously established conventions [43], females ranked 1 or 2 in their group were classified as dominant and those ranked 3, 4, or 5 were considered subordinate. This categorization yielded 8 dominant and 12 subordinate females. Behavioral observations to confirm dominance ranks were quantified from two to three-weekly 30-min sessions in which all occurrences of aggressive and submissive behavior were recorded following previously described procedures [25]. The frequency of each behavior during each 30-min test was summarized and multiplied by 2 to obtain hourly rates. Average rates of behavior across the tests were obtained for each female.

The study duration was 12 weeks and was comprised of two 4-week treatment intervals with a 4-week washout period separating the treatment phases. Half of the animals ($n = 10$) received the 4-week non-leptin treatment condition followed by a 4-week "washout" at which time they were treated with leptin for 4 weeks. The remaining animals ($n = 10$) received the treatments in the same order but their control phase coincided with the leptin treatment condition for the first set (January). As there was no reason to suspect that the non-leptin treatment would confound the effects of the leptin treatment, particularly with a 4-week "rest' period in-between, the study was designed to control for time of the year because these animals were housed in indoor–outdoor runs.

All females were replaced with low-dose estradiol (E2) throughout the study to maintain serum concentrations at early follicular phase levels. Estradiol was administered by implanting Silastic-filled capsules filled with crystalline E2 (Sigma Chemical Corporation, St. Louis, MO, USA) to achieve circulating E2 levels equivalent to mid-follicular phase levels [94]. Capsules were implanted subcutaneously between the scapulas while the animals were anesthetized (10 mg/kg, ketamine hydrochloride, Henry Schein). The "Control" condition consisted of no leptin supplementation while the treatment consisted of infusion of leptin. Recombinant human leptin (Sigma Chemical Corporation) was delivered at a dose of 6 μg/kg/day for 28 days by constant infusion with an osmotic mini-pump (Alzet 2ML4; Durret Corporation, Cupertino, CA, USA) implanted subcutaneously between the scapulas during ketamine anesthesia. This is equivalent to the dose of human leptin used previously in monkeys to evaluate leptin effects of LHPA responsivity [33]. Chronic administration of human leptin to rhesus monkeys induces non-neutralizing antibodies that sequesters leptin in circulation and elevates serum concentrations. This does not reflect leptin resistance or a diminution in leptin bioactivity, as levels of GH are consistently elevated in leptin compared with non-leptin-treated animals in the presence of these antibodies [95, 96]. Furthermore, this same treatment regimen with human leptin administered to juvenile monkeys for 12 months advances the onset of puberty [96].

The consequences of the treatments as a function of social status on LHPA responsivity (cortisol) were evaluated in three ways. Effects on the diurnal cortisol rhythm were determined in serum samples collected at 0900, 1200, and 1800 hours on day 15 of each treatment phase. Secondly, effects on glucocorticoid negative feedback were determined by using a dexamethasone suppression test on days 15 and 16 of each phase. Following the collection of a serum sample at 1800 hours, dexamethasone (0.25 mg/kg, IM; American Regent Inc., Shirley, NY, USA) was administered and subsequent samples were collected at 0900 and 1200 hours on day 16, corresponding to 15, 18, and 19 hours following the injection. This dose of dexamethasone has been used previously to show social status differences in glucocorticoid negative feedback in macaques [97–99]. Finally, we used a modification of a social separation paradigm. Removal of a monkey from the group to a novel location is a potent stressor that activates the LHPA axis [64]. Because monkeys are xenophobic [39], we removed a female from her group for the social separation test to a single cage in another room at the facility that contained unfamiliar rhesus monkeys. The test was conducted on day 10 or 11 of each phase. On the day of the test, the female was removed from the group at 0930 hours and a serum sample collected. The female was then taken to a room containing four singly housed unfamiliar female rhesus monkeys, and placed in a single cage. At the end of 30 min, a second serum sample was obtained and the female was returned to her home group. To control for the repeated sampling, a non-separation control condition was also included. On day 7 of each phase, a female was removed from her group at 0930 hours for the collection of a serum sample after which she was returned to her group. A second sample was collected 30 min later.

All females had been habituated to brief removal from their group for the collection of blood samples without

anesthesia. These procedures are well validated [100] and have no adverse effect on behavior and a number of physiological systems [96, 101–103]. However, to minimize any effect the procedure may have on the LHPA axis, every attempt was made to obtain the blood samples within 10 min of the staff entering the animal housing area. In addition to the samples collected for LHPA activity described above, additional samples for estradiol and leptin analyses were obtained on days 1, 3, 5, 14, and 21. Non-fasted body weights were obtained weekly. BMI was also calculated (weight in kilograms divided height in m^2). Height and SAD were obtained while the animals were anesthetized (ketamine; 5 mg/kg; Henry Schein) as previously described [25]. Heights (cm) did not vary among the four groups (dominant: 76.3 ± 0.7; subordinate: 76.7 ± 0.7).

Serum cortisol was measured by radioimmunoassay using commercially available reagents (Beckman-Coulter/DSL Labs, Webster, TX, USA). The assay has a sensitivity of 0.50 μg/dl and an inter- and intra-assay coefficient of variation (CV) of 8.2 and 3.1%, respectively. Serum E2 was measured in selected samples to confirm efficacy of E2 treatments. The previously validated radioimmunoassay [104] is a modification of a commercially available kit (Siemens/DPC, Los Angeles, CA, USA) having a sensitivity of 2.5 pg/ml and an inter- and intra-CV of 10.2 and 6.5%, respectively. Group differences in endogenous leptin, as well as the efficacy of the leptin treatments, were determined by radioimmunoassay using a primate specific kit from Linco Inc. (St. Louis, MO, USA). The assay has a sensitivity of 0.5 ng/ml and an inter- and intra-CV of 9.2 and 5.6%, respectively.

All data were expressed as mean ± SEM and were analyzed using analysis of variance models for repeated measures. Non-repeated main effects were social status (dominant versus subordinate), whereas repeated measures were estradiol and leptin treatments, as well as time. Significant interactions were further evaluated using Fisher post-hoc tests using the Bonferroni correction to adjust the *P* value. If the assumption of homogeneity of variance was violated, data were $\log_{10}$ transformed.

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References

1. J.R. Kaplan, S.B. Manuck, T.B. Clarkson, F.M. Lusso, D.T. Taub, E.W. Miller, Science **220**, 733 (1983)
2. A. Rozanski, J.A. Blumenthal, J.R. Kaplan, Circulation **99**, 2192 (1999)
3. T.L. Bale, Horm. Behav. **50**, 529 (2006)
4. C.S. de Kloet, E. Vermetten, E. Geuze, A. Kavelaars, C.J. Heijnen, H.G. Westenberg, J. Psychiatr. Res. **40**, 550 (2006)
5. S.A. French, M. Story, D. Neumark-Sztainer, B. Downes, M. Resnick, R. Blum, Int. J. Eat. Disord. **22**, 315 (1997)
6. Y. Ueta, Y. Ozaki, J. Saito, T. Onaka, Exp. Biol. Med. **228**, 1168 (2003)
7. S. Boullu-Ciocca, A. Dutour, V. Guillaume, V. Achard, C. Oliver, M. Grino, Diabetes **54**, 197 (2005)
8. P. Bjorntorp, G. Holm, R. Rosmond, Diabet. Med. **16**, 373 (1999)
9. G. Koob, M.J. Kreek, Am. J. Psychiatry **164**, 1149 (2007)
10. S.L. Berga, T.L. Loucks, Minerva Ginecol. **57**, 45 (2005)
11. R.M. Sapolsky, Semin. Neurosci. **6**, 261 (1994)
12. G.E. Miller, A.K. Ritchey, S. Cohen, Health Psychol. **21**, 531 (2002)
13. A.A. Zozulya, M.V. Gabaeva, O.Y. Sokolov, I.D. Surkina, N.V. Kost, J. Immunotoxicol. **5**, 221 (2008)
14. D.B. Miller, J.P. O'Callaghan, Metab. Clin. Exp. **51**, 5 (2002)
15. S. Bhatnagar, M. Dallman, Neuroscience **84**, 1025 (1998)
16. D.S. Albeck, C.R. McKittrick, D.C. Blanchard, R.J. Blanchard, J. Nikulina, B.S. McEwen, R.R. Sakai, J. Neurosci. **17**, 4895 (1997)
17. M.F. Dallman, N. Pecoraro, S.F. Akana, S.E. La Fleur, F. Gomez, H. Houshyar, M.E. Bell, S. Bhatnagar, K.D. Laugero, S. Manalo, Proc. Natl Acad. Sci. USA **100**, 11696 (2003)
18. J.D. Shepard, K.W. Barron, D.A. Myers, Brain Res. **861**, 288 (2000)
19. S.C. Stout, P. Mortas, M.J. Owens, C.B. Nemeroff, J. Moreau, Eur. J. Pharmacol. **401**, 39 (2000)
20. M. Dallman, S. Bhatnagar, in *Handbook of Physiology, Section 7: The Endocrine System*, ed. by B. McEwen (Oxford University Press, New York, 2001)
21. G.N. Smagin, L.A. Howell, S. Redmann Jr., D.H. Ryan, R.B. Harris, Am. J. Physiol. **276**, R1461 (1999)
22. V.P. Bakshi, S.M. Newman, S. Smith-Roe, K.A. Jochman, N.H. Kalin, J. Neurosci. **27**, 10568 (2007)
23. H.L. Fehm, W. Kern, A. Peters, Prog. Brain Res. **153**, 129 (2006)
24. K.L. Tamashiro, M.M. Nguyen, T. Fujikawa, T. Xu, L. Yun Ma, S.C. Woods, R.R. Sakai, Physiol. Behav. **80**, 683 (2004)
25. H. Jarrell, J.B. Hoffman, J.R. Kaplan, S. Berga, B. Kinkead, M.E. Wilson, Physiol. Behav. **93**, 807 (2008)
26. L.K. Malendowicz, M. Rucinski, A.S. Belloni, A. Ziolkowska, G.G. Nussdorfer, Int. Rev. Cytol. **263**, 63 (2007)
27. C.D. Walker, Prog. Neuropsychopharmacol. Biol. Psychiatry **29**, 1249 (2005)
28. A. Giovambattista, A.N. Chisari, R.C. Gaillard, E. Spinedi, Neuroendocrinology **72**, 341 (2000)
29. M.L. Heiman, R.S. Ahima, L.S. Craft, B. Schoner, T.W. Stephens, J.S. Flier, Endocrinology **138**, 3859 (1997)
30. M. Oates, B. Woodside, C.D. Walker, Horm. Behav. **37**, 366 (2000)
31. A. Kawakami, N. Okada, K. Rokkaku, K. Honda, S. Ishibashi, T. Onaka, Stress **11**, 363 (2008)
32. K.W. Nowak, K. Pierzchala-Koziec, C. Tortorella, G.G. Nussdorfer, L.K. Malendowicz, Int. J. Mol. Med. **9**, 61 (2002)
33. M.E. Wilson, J. Fisher, J. Brown, Physiol. Behav. **84**, 449 (2005)
34. T.L. Garthwaite, D.R. Martinson, L.F. Tseng, T.C. Hagen, L.A. Menahan, Endocrinology **107**, 671 (1980)
35. Q. Huang, R. Rivest, D. Richard, Endocrinology **139**, 1524 (1998)
36. K. Arvaniti, Q. Huang, D. Richard, Neuroendocrinology **73**, 227 (2001)
37. K.L. Tamashiro, M.M. Nguyen, R.R. Sakai, Front. Neuroendocrinol. **26**, 27 (2005)

38. I.S. Bernstein, J. Theor. Biol. **60**, 459 (1976)
39. I.S. Bernstein, T.P. Gordon, Am. Sci. **62**, 304 (1974)
40. I.S. Bernstein, T.P. Gordon, R.M. Rose, Folia Primatol. **21**, 81 (1974)
41. C. Shively, J. Kaplan, Physiol. Behav. **33**, 777 (1984)
42. D.H. Abbott, E.B. Keverne, F.B. Bercovitch, C.A. Shively, S.P. Mendoza, W. Saltzman, C.T. Snowdon, T.E. Ziegler, M. Banjevic, T. Garland, R.M. Sapolsky, Horm. Behav. **43**, 67 (2003)
43. J.R. Kaplan, M.R. Adams, T.B. Clarkson, D.R. Koritnik, Atherosclerosis **53**, 283 (1984)
44. S. Cohen, Ann. N. Y. Acad. Sci. **896**, 246 (1999)
45. M.E. Wilson, J. Fisher, A. Fischer, V. Lee, R.B. Harris, T.J. Bartness, Physiol. Behav. **94**, 586 (2008)
46. M.E. Wilson, K. Pazol, A. Legendre, J. Fisher, K. Chikazawa, Endocrine **26**, 89 (2006)
47. M. Paiardini, J. Hoffman, B. Cervasi, A.M. Ortiz, F. Stroud, G. Silvestri, M.E. Wilson, Brain Behav. Immun. **23**, 286 (2009)
48. C.A. Shively, K. Laber-Laird, R.F. Anton, Biol. Psychiatry **41**, 871 (1997)
49. C.A. Shively, Biol. Psychiatry **44**, 882 (1998)
50. J.R. Kaplan, M.R. Adams, T.B. Clarkson, S.B. Manuck, C.A. Shively, J.K. Williams, Psychosom. Med. **58**, 598 (1996)
51. D. Morgan, K.A. Grant, H.D. Gage, R.H. Mach, J.R. Kaplan, O. Prioleau, S.H. Nader, N. Buchheimer, R.L. Ehrenkaufer, M.A. Nader, Nat. Neurosci. **5**, 169 (2002)
52. K.A. Grant, C.A. Shively, M.A. Nader, R.L. Ehrenkaufer, S.W. Line, T.E. Morton, H.D. Gage, R.H. Mach, Synapse **29**, 80 (1998)
53. C.A. Shively, D.P. Friedman, H.D. Gage, M.C. Bounds, C. Brown-Proctor, J.B. Blair, J.A. Henderson, M.A. Smith, N. Buchheimer, Arch. Gen. Psychiatry **63**, 396 (2006)
54. J.R. Kaplan, S.B. Manuck, ILAR **45**, 89 (2004)
55. D.A. Gust, T.P. Gordon, M.E. Wilson, A. Ahmed-Ansari, A.R. Brodie, H.M. McClure, Brain Behav. Immun. **5**, 296 (1991)
56. C.A. Shively, T.C. Register, D.P. Friedman, T.M. Morgan, J. Thompson, T. Lanier, Biol. Psychol. **69**, 67 (2005)
57. A. Troisi, Stress **5**, 47 (2002)
58. C.A. Czeisler, E.B. Klerman, Recent Prog. Horm. Res. **54**, 97 (1999)
59. R.E. Dahl, Semin. Pediatr. Neurol. **3**, 44 (1996)
60. A. Sadeh, R. Gruber, A. Raviv, Child Dev. **73**, 405 (2002)
61. A. Oskis, C. Loveday, F. Hucklebridge, L. Thorn, A. Clow, Psychoneuroendocrinology **34**, 307 (2009)
62. S. Bhatnagar, C. Vining, Horm. Behav. **43**, 158 (2003)
63. C.M. Berman, K.L. Rasmussen, S.J. Suomi, Child Dev. **65**, 1028 (1994)
64. D.A. Gust, T.P. Gordon, M.K. Hambright, Physiol. Behav. **53**, 599 (1993)
65. S.R. Bornstein, K. Uhlmann, A. Haidan, M. Ehrhart-Bornstein, W.A. Scherbaum, Diabetes **46**, 1235 (1997)
66. F.P. Pralong, R. Roduit, G. Waeber, E. Castillo, F. Mosimann, B. Thorens, R.C. Gaillard, Endocrinology **139**, 4264 (1998)
67. H.T. Hsu, Y.C. Chang, Y.N. Chiu, C.L. Liu, K.J. Chang, I.C. Guo, J. Clin. Endocrinol. Metab. **91**, 2761 (2006)
68. M. Sone, R.Y. Osamura, Pituitary **4**, 15 (2001)
69. J. Harvey, J. Neurochem. **100**, 307 (2007)
70. S. Diano, S.P. Kalra, H. Sakamoto, T.L. Horvath, Brain Res. **812**, 256 (1998)
71. P.D. Finn, M.J. Cunningham, D.G. Rickard, D.K. Clifton, R.A. Steiner, J. Clin. Endocrinol. Metab. **86**, 422 (2001)
72. S. Fulton, P. Pissios, R.P. Manchon, L. Stiles, L. Frank, E.N. Pothos, E. Maratos-Flier, J.S. Flier, Neuron **51**, 811 (2006)
73. J.G. Mercer, K.M. Moar, N. Hoggard, Endocrinology **139**, 29 (1998)
74. J.T. Smith, B.V. Acohido, D.K. Clifton, R.A. Steiner, J. Neuroendocrinol. **18**, 298 (2006)
75. J. Harvey, N. Solovyova, A. Irving, Prog. Lipid Res. **45**, 369 (2006)
76. X.Y. Lu, C.S. Kim, A. Frazer, W. Zhang, Proc. Natl Acad. Sci. USA **103**, 1593 (2006)
77. J.D. Gardner, N.J. Rothwell, G.N. Luheshi, Nat. Neurosci. **1**, 103 (1998)
78. M. Nikodemova, C.R. Diehl, G. Aguilera, Arch. Physiol. Biochem. **110**, 123 (2002)
79. K.J. Kovacs, A. Fioldes, P.E. Sawchenko, J. Neurosci. **20**, 3843 (2000)
80. J.P. Herman, D. Adams, C. Prewitt, Neuroendocrinology **61**, 180 (1995)
81. K. Proulx, S. Clavel, G. Nault, D. Richard, C.D. Walker, Endocrinology **142**, 4607 (2001)
82. M.P. Howell, L.J. Muglia, Front. Neuroendocrinol. **27**, 275 (2006)
83. M.P. Warren, F. Voussoughian, E.B. Geer, E.P. Hyle, C.L. Adberg, R.H. Ramos, J. Clin. Endocrinol. Metab. **84**, 873 (1999)
84. A.J. Tilbrook, E.A. Rivalland, A.I. Turner, G.W. Lambert, I.J. Clarke, Neuroendocrinology **87**, 193 (2008)
85. M.E. Gluck, A. Geliebter, M. Lorence, Ann. N. Y. Acad. Sci. **1032**, 202 (2004)
86. C.D. Morrison, Physiol. Behav. **94**, 660 (2008)
87. O. Johren, A. Dendorfer, P. Dominiak, W. Raasch, Brain Res. **1184**, 160 (2007)
88. M.F. Dallman, N.C. Pecoraro, S.E. La Fleur, Brain Behav. Immun. **19**, 275 (2005)
89. K.L. Tamashiro, M.A. Hegeman, R.R. Sakai, Physiol. Behav. **89**, 536 (2006)
90. S.E. La Fleur, H. Houshyar, M. Roy, M.F. Dallman, Endocrinology **146**, 2193 (2005)
91. N. Pecoraro, F. Reyes, F. Gomez, A. Bhargava, M.F. Dallman, Endocrinology **145**, 3754 (2004)
92. M.T. Foster, J.P. Warne, A.B. Ginsberg, H.F. Horneman, N.C. Pecoraro, S.F. Akana, M.F. Dallman, Endocrinology **150**, 2325 (2009)
93. J.B. Hoffman, J.R. Kaplan, B. Kinkead, S.L. Berga, M.E. Wilson, Endocrine **31**, 202 (2007)
94. M.E. Wilson, T.P. Gordon, J.M. Tanner, J. Endocrinol. **137**, 519 (1993)
95. M.L. Barker-Gibb, A. Sahu, C.R. Pohl, T.M. Plant, J. Clin. Endocrinol. Metab. **87**, 4976 (2002)
96. M.E. Wilson, J. Fisher, K. Chikazawa, R. Yoda, A. Legendre, D. Mook, K.G. Gould, J. Clin. Endocrinol. Metab. **88**, 4874 (2003)
97. M.E. Wilson, A. Legendre, K. Pazol, J. Fisher, K. Chikazawa, Endocrine **26**, 89 (2005)
98. S.M. Brooke, A.M. de Haas-Johnson, J.R. Kaplan, S.B. Manuck, R.M. Sapolsky, Neuroendocrinology **60**, 134 (1994)
99. C.A. Shively, J.K. Williams, K. Laber-Laird, R.F. Anton, Psychosom. Med. **64**, 699 (2002)
100. M.S. Blank, T.P. Gordon, M.E. Wilson, Acta Endocrinol. **102**, 190 (1983)
101. M.L. Walker, T.P. Gordon, M.E. Wilson, J. Med. Primatol. **11**, 291 (1982)
102. M.L. Walker, M.E. Wilson, T.P. Gordon, Endocrinology **114**, 1074 (1984)
103. K. Wallen, P.L. Tannenbaum, Ann. N. Y. Acad. Sci. **807**, 185 (1997)
104. K. Pazol, J.R. Kaplan, D. Abbott, S.E. Appt, M.E. Wilson, Clin. Chim. Acta **340**, 117 (2004)