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Body weight decreases induced by estradiol in female rhesus monkeys are dependent upon social status

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Abstract

Gonadal steroids regulate appetite and thus body weight. In addition, continuous exposure to stressors negatively influences appetite through circuits likely distinct from those of gonadal steroids. The occurrence of adverse metabolic consequences due to chronic exposure to psychosocial stressors is twice as frequent in women as men, implicating a role for ovarian hormones, estradiol (E2) and progesterone (P4), in modulating stress-induced changes in appetite. Using social subordination in female macaques as a model of social stress, the current study tested the hypothesis that subordinate females would lose more weight during E2 treatment and gain less weight during P4 administration than dominant females. Because polymorphisms in the gene encoding the serotonin transporter (5HTT; *SCL6A4*) are known to alter responsivity to stress, we hypothesized that weight loss during E2 administration would be greatest in females with the short variant (s-variant) allele of 5HTT. Dominant females were significantly heavier than subordinate animals throughout the study, a result consistent with previous accounts of food intake when animals are fed a low-fat, high-fiber diet. Females with the s-variant 5HTT genotype weighed significantly less than l/l animals. Dominant animals lost significantly more weight than subordinate animals during E2 treatment. Administration of P4 blocked the weight-reducing effects of E2 in all females, regardless of social status. These data provide evidence that social subordination modulates the influence of ovarian steroid hormones on body weight in female rhesus monkeys independent of 5HTT genotype. Given the prosocial effects of these steroids, future studies are necessary to determine whether status differences in E2-induced weight loss are due to diminished food intake and/or increases in energy expenditure and how the change in energy availability during E2 treatments relates to a female's motivation to interact with conspecifics.

Keywords

social subordination; psychosocial stress; body weight; estrogen; progesterone; metabolism

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Introduction

Rhesus macaque females, when housed socially, organize themselves into a linear dominance hierarchy that is enforced by the constant harassment and threat of aggression towards subordinate members by more dominant animals [1,2]. Subordinate females show reductions in body weight and metabolic hormones [3,4], including reduced fat mass and circulating leptin concentrations in comparison to dominant animals [5] when fed a standard low-calorie monkey chow [6]. These metabolic alterations are accompanied by disruptions in limbic-hypothalamic-pituitary-adrenal axis (LHPA) activity, as subordinate females are hypercortisolemic due to diminished glucocorticoid negative feedback [4,7,8]. Heightened activity of corticotropin-releasing hormone (CRH) in rodents mediates the anorectic effects of exposure to chronic stressors [9–11] and could be the underlying cause of reductions in body weight seen in subordinate animals.

The occurrence of adverse metabolic consequences due to chronic psychosocial stressors is more prevalent in women than in men, as women are twice as susceptible than men to developing eating disorders and altered metabolism [12–14]. This increased vulnerability in women implicates the role of the ovarian steroid hormones, estradiol (E2) and progesterone (P4), in affecting body weight and metabolism. Indeed, E2 replacement and high estrogen levels associated with stages of the ovarian cycle are linked to decreases in body weight [15] and food intake [16–19] in rodents and humans. Periods of elevated P4 coincide with increases in food intake and body weight in women [15,19–21] and synthetic progestins are known to increase body weight [22]. While the effects of E2 and P4 on body weight are well described, it is unclear how the effects of social stress interact with those of gonadal steroids to regulate body weight.

Individual vulnerability to adverse health outcomes associated with chronic exposure to stressors is likely affected by a number of genetic factors. In particular, polymorphisms in the gene (*SCL6A4*) that encodes the serotonin transporter (5HTT) [23] are associated with greater vulnerability to environmental stressors in monkeys and in people. The short promoter length variant (s-variant) of 5HTT has reduced transcriptional activity in humans [24] and individuals with this short promoter allele have a higher incidence of psychopathology [23] than individuals homozygous for the long 5HTT allele (l/l). Rhesus monkeys also show homologous polymorphisms in 5HTT [25,26] and the s-variant allele increases individual susceptibility to adverse effects of social subordination on reproduction and metabolism [5,27].

The present study was designed to determine how social stress, resulting from social subordination, interacts with gonadal steroids to influence body weight in females. Using adult ovariectomized rhesus monkeys, we hypothesized that replacement therapy with E2 would produce the greatest decrements in body weight in subordinate compared with more dominant animals and that this effect would be exacerbated by the short promoter length allele in the 5HTT gene. Because progestins promote weight gain, the study determined whether administration of P4 would attenuate the effects of E2.

Methods

Animals

Subjects included 39 ovariectomized adult female rhesus monkeys (13–17 years old) socially housed in indoor-outdoor runs in small social groups without males ($n = 4$ or 5 per group) as previously described [5]. Animals were fed standard monkey chow (low-fat, high-fiber diet from PMI Lab Diets, #5038, St. Louis MO) *ad libitum* twice-daily via two chow bins from which multiple animals could access food simultaneously. We observe no

evidence of more dominant monkeys restricting subordinate females from access to food under these ad libitum conditions, even when more preferred diets are available [4]. In addition, animals had continuous access to water and received daily supplementation with seasonal fresh fruit and vegetables. All females had been previously ovariectomized 18 months prior to initiation of the study and genotyped for 5HTT gene polymorphism, either having two copies of the long promoter length alleles (l/l) or having at least one short promoter length allele (s-variant) [28]. Groups were homogeneous for genotype such that four groups were comprised of all females with the l/l genotype and 4 groups entirely of the s-variant genotype [5]. Dominance status was assessed by performing 30 min group scans of aggressive and submissive behaviors twice weekly throughout each of the 4-week treatments. Subordinate status was determined by the unequivocal submissive behavior emitted by a female to a group mate [29]. Data were recorded using a Palm PDA and the “Hands Obs” program developed by the Center for Behavioral Neuroscience [30]. Inter-observer reliability exceeded 90%. Females ranked 1 and 2 were considered as dominant and females ranked 3–5 were classified as subordinate [7]. Total numbers of animals classified by status and genotype were: 8 dominant, l/l; 8 dominant, s-variant; 11 subordinate, l/l, and 12 subordinate, s-variant. Groups had been formed for 12 months prior to the initiation of this study. All procedures were approved by the Emory University Animal Care and Use Committee in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services “Guide for Care and Use of Laboratory Animals.”

Treatment Conditions

Animals were subjected to four different hormonal manipulations each lasting four weeks in duration. The four treatment conditions were control (C) followed immediately by E2 replacement (E2), and progesterone replacement (P4) followed immediately by E2 plus P4 (E2 + P4). The order (C – E2) and P4 – E2+P4) was randomized among the females but counterbalanced to ensure half the females received the C condition first and the other half the P4 condition first. A two-week washout period separated the first treatment sequence from the second. E2 and P4 replacement was attained via Silastic capsules implanted subcutaneously between the scapulae as previously described [31]. Analyses of blood samples collected on days 5 and 26 of each of the four treatment conditions showed that, compared to the control condition, hormone replacement achieved mid-follicular phase levels of E2 (49.7 ± 15.8 vs. <5.0 pg/ml) and typical luteal phases concentrations for P4 (4.29 ± 0.11 vs. 0.61 ± 0.03 ng/ml).

Outcome measures

All blood samples were taken within 10 minutes from disturbance time via conscious venipuncture using previously validated methods to minimize arousal [32,33]. Fasted blood samples were collected at the end of each four-week treatment period between 0800 and 0900 hours, some 10 hours after food have been removed from their food bins. Blood was centrifuged and serum frozen for subsequent analyses. Leptin was assessed as a peripheral index of adiposity and acute energy balance [34,35]. Peripheral insulin and glucose were measured as signals of glucose regulation [36]. Weekly body weights were collected and sagittal abdominal circumference (SAC) [37] was taken at the end of each four-week treatment period. SAC was determined by measuring the circumference of the abdomen at the level of the navel while animals were in the supine position during anesthesia with ketamine (10 mg, kg, IM) [5]. Two independent measures of SAC were measured and the mean value calculated for each time point.

Hormone assays

All assays were performed in the Biomarkers Core Lab at the Yerkes National Primate Research Center (YNPRC). Selected samples were assayed for E2 to verify Silastic capsule efficacy using a modification of a previously validated commercial RIA from Diagnostics Products Corporation (DPC, Los Angeles, CA) [38]. Using 200 μ l of serum, the assay has a sensitivity of 5 pg/ml and an intra- and inter-assay coefficient of variation (CV) of 5.2% and 11.1%, respectively. Progesterone levels were determined using a modification of a previously described RIA using a commercially available kit (Siemens) [39]. Briefly, 125 μ l of sample are extracted with anesthesia grade ether and the organic layer evaporated off by a stream of N₂. The sample is reconstituted in 125 μ l of the assay buffer and replicates are assayed following the kit protocol. The sensitivity of the assay is 0.10 ng/ml with an inter- and intra-assay CV of 8.14% and 7.73%, respectively. Serum leptin was measured by a RIA using a commercially available kit (Millipore, St. Louis MO). Assaying 100 μ l, the assay has a range of 0.5 to 100 ng/ml. Intra-assay CVs were 6.84% and inter-assay were 7.24% [39]. Serum insulin was assayed with a RIA kit from Siemens having a sensitivity of 3 to 372 IU/L and an inter- and intra-assay CV of 9.02% and 5.87%, respectively [5]. Serum glucose was determined by a commercially available colorimetric enzyme assay (Stanbio Laboratory, Boerne TX), having a range from 0 to 27 mmol/L and inter- and intra-assay CVs of 2.12% and 4.21%, respectively [5].

Statistical analyses

Data were summarized as mean $\pm$ standard error of the mean (SEM) using PASW SPSS v18 (IBM, Somers NY). An analysis of variance for repeated measures was used to assess the main effects of the between-subject factors status (dominant vs. subordinate) and 5HTT genotype (l/l vs. s-variant), and the within-subject factor of the four treatment conditions (C, E2, P4, and E2+P4), as well as their interactions on overall body weight, SAC, leptin, glucose and insulin. A similar analysis was undertaken to assess the change in body weight and SAC over the course of each 4-wk hormonal condition (final value minus initial value on each hormone condition). If the main effect of treatment or status - genotype interactions with treatment were significant, simple main effects were evaluated with the Newman-Keuls test to isolate specific group or treatment effects. Such corrections were unnecessary for main effects of status and genotype as each had only two levels.

Results

Social Status Categorization

Figure 1 shows rates of aggression received and submissive behavior emitted by monkeys at each social dominance rank position, independent of 5HTT genotype. These data were derived from two-30 min group scans of aggressive and submissive behaviors obtained each week of the four treatment conditions. Hormone treatments did not alter the dominance hierarchy in any of the groups. As expected, subordinate females (ranks 3 – 5) received significantly more aggression from higher ranking cage mates ($F_{1,8} = 10.6$, $p = 0.003$) and, consequently, emitted significantly more submissive behaviors ($F_{1,8} = 17.9$, $p < 0.001$).

Body Weight and Sagittal Abdominal Circumference

Body weight and SAC throughout the 16-week study were consistently greater in dominant females than subordinate animals ($F_{1,35} = 5.59$, $p = 0.02$ and $F_{1,35} = 6.60$, $p = 0.02$ respectively) (Table 1). There was also a significant main effect of genotype, as body weights ($F_{1,35} = 4.91$, $p = 0.03$) and SAC ($F_{1,35} = 5.59$, $p = 0.02$) were significantly greater in l/l compared with s-variant females (Table 1). In addition, the effect of social status on SAC, but not on body weight ($F_{1,35} = 2.32$, $p = 0.14$), was modified significantly by 5HTT

genotype ($F_{1,35} = 5.10$, $p=0.03$). Post hoc analyses revealed that dominant I/I females had significantly greater SAC ($p < 0.03$) than any of the other groups of females who did not differ from one another ($p > 0.05$; Table 1).

To assess the effect of treatment on body weight, the change in weight from day 0 to day 28 of each treatment condition was calculated. As illustrated in Figure 2A, the change in body weight was significantly affected by treatment ($F_{3,105} = 15.68$, $p<0.001$). Post hoc analyses revealed this main effect of treatment was due to a significant loss of body weight during E2 administration compared to an increase in body weight during the other three treatments ($p < 0.001$). However, administration of E2 in combination with P4 did not decrease body weight, as weights were similar to the P4 alone and control treatments ($p > 0.05$).

This effect of treatment on the change in body weight was modified by the interaction of social status (Figure 2A; $F_{3,105} = 3.46$, $p=0.02$). Post hoc analyses revealed that dominant animals lost significantly more weight than subordinate animals on E2 alone ($F_{1,35} = 4.45$, $p=0.04$). Body weight changes during the control, P4, or E2 + P4 conditions were unaffected by status ($p > 0.05$). 5HTT genotype did not interact with treatment ($F_{1,35} = 0.76$, $p=0.39$) or status and treatment ($F_{1,35} = 0.06$, $p=0.81$) to affect the change in body weight.

Parallel to these changes in body weight were changes in waist circumference as illustrated in Figure 2B. The significant effect of treatment on changes in SAC ($F_{3,105} = 10.49$, $p<0.001$) was again accounted for by E2 administration. Post hoc analyses revealed SAC decreased significantly during E2 compared with the other treatment conditions ($p < 0.01$). The increase in SAC during control, P4, and E2+P4 did not vary significantly ($p > 0.05$). Finally, these treatment-dependent changes in SAC were not influenced by status ($F_{1,35} = 0.12$, $p=0.73$) or 5HTT genotype ($F_{1,35} = 1.52$, $p=0.23$).

Metabolic measures

Dominant females had higher overall serum leptin levels than subordinate females throughout the duration of the study ($F_{1,35} = 4.46$, $p=0.04$, Table 2). Leptin levels did not vary significantly with 5HTT genotype or by a status by genotype interaction ($F_{1,35} = 1.59$, $p=0.22$ and $F_{1,35} = 0.16$, $p=0.69$ respectively). Even though changes in SAC were influenced by E2 treatment, there were no significant effects on these hormonal manipulations on changes in serum leptin levels ($F_{3,105} = 1.08$, $p = 0.36$). However, treatment condition significantly affected serum insulin ($F_{3,105} = 6.70$, $p<0.001$) but not serum glucose ($F_{3,105} = 2.09$, $p=0.11$). Post hoc analyses revealed that E2 administration significantly reduced serum insulin compared to the other treatment conditions ($p=0.03$). While it appeared that administration of P4 increased serum insulin compared to other conditions, the difference was not significant ($p>0.05$). These treatment effects on serum insulin were not affected by status ($F_{3,105} = 0.33$, $p=0.81$) or genotype ($F_{3,105} = 0.47$, $p=0.70$). Similarly, serum glucose was also unaffected by status ($F_{3,105} = 2.07$, $p=0.159$) or genotype ($F_{3,105} = 1.024$, $p=0.318$; Table 2).

Discussion

Subordinate females, receiving more aggression and emitting more submissive behavior, maintained lower body weights, abdominal adiposity, and peripheral leptin than dominant females. Furthermore, as observed previously [5], dominant females with an I/I 5HT genotype were heavier than dominant s-variant females throughout the study period. These status differences in body weight are consistent with previous reports of status-dependent body weight differences [5,40] due to diminished food intake in female rhesus monkeys [6]. Importantly, E2 administration significantly lowered body weight and estimates of body fat in all females but the effect on body weight was modulated by social status, as dominant

females, regardless of 5HTT genotype, lost more body weight than did subordinate females. However, co-administration of P4 with E2 blocked the effects of E2 on body weight and fat. Taken together, these data indicate that social status modulates the effects of E2 on body weight.

Even though food intake and energy expenditure were not monitored in the current study, the overall status difference in body weights and abdominal adiposity is consistent with our previous observation that subordinate animals consume fewer calories daily than dominant animals when only a standard low-calorie diet is available [6]. Disruptions of food intake due to heightened activity of the LHPA axis that is characteristic of chronic stress conditions in humans and rodents, as well as social subordination in macaques, are dependent upon diet type and availability [4,9,10,13,14,41]. Chronic LHPA activity in rodents and monkeys decreases food intake only when a low-calorie standard chow is available, an effect due to the anorectic action of CRH – urocortin system [5,10,42–44].

The administration of ovarian steroid hormones differentially altered body weight and sagittal adiposity over the course of a four-week treatment. As expected, replacement of E2 alone decreased body weight and sagittal adiposity in all groups of females. The decrease in body weight seen during E2 replacement corroborates previous reports that E2 attenuates body weight in monkeys and rodents [17,18] and the inverse correlation between E2 and body weight over the course of the menstrual cycle in women [15]. Even though we did not assess food intake during the course of this study, ample literature suggests that E2 is anorectic [19] and attenuates body weight primarily by reducing meal size [45]. E2 modulates the expression of molecules crucial to feeding behavior at the level of the hypothalamus by decreasing the orexigenic effects of melanin-concentrating hormone (MCH, [46]), neuropeptide Y (NPY [47]), and ghrelin [48]. E2 also acts at the level of the nucleus of the solitary tract to have anorectic effects via estrogen receptor alpha (ER α) activation [19,49].

We had hypothesized that E2 would interact with status to produce a greater loss in subordinates. While E2 decreased body weight in all females, dominant females lost significantly more weight than did subordinates. Despite the greater weight loss by dominant females under E2 administration, subordinate animals nonetheless still weighed significantly less. These data imply that the anorectic actions of E2 are related to preexisting energy stores represented by body weight and waist circumference. Without measuring food intake [19] or energy expenditure [50], we can only hypothesize that dominant females are more sensitive to these anorectic actions of estradiol. The mechanisms that would reduce the anorectic action of E2 in females with reduced body weights, which in the present study are subordinate females, are unknown and we are unaware of any studies that have assessed this relation. It is possible that signals from the LHPA axis attenuate the action of E2. For example, stressor exposure [51] or CRH over-expression in the amygdala [52] attenuate E2 activation of female sexual behavior. In any event, it is possible that even the smaller weight loss in subordinate females due to E2 has put even greater demands on their energy balance that could affect other homeostatic processes. Indeed, previous studies of female rhesus monkeys show subordinates are hypersensitive to the negative feedback action of low dose E2 treatment on LH (luteinizing hormone) secretion [53], an observation that could be due to the direct effects of E2 on the GnRH (gonadotropin-releasing hormone) – gonadotropic axis or to deficits in metabolic energy necessary to support reproductive function. Thus, although the weight loss induced by E2 may be less in subordinate females, the functional consequences may nonetheless be greater compared with dominant animals.

Administration of P4 in combination with E2 negated E2-induced weight loss and increased body weight in all females compared to the E2 alone condition. Periods of high P4,

including the luteal phase of the menstrual cycle and pregnancy, coincide with increases in food intake and body weight [15,19–21]. Changes in orexigenic signals such as NPY and agouti-related protein (AgRP) are expressed at these times of maximal P4 circulation [54–56]. The ability of P4 to increase food intake could be due to changes in gene expression mediated by activation of the progesterone receptor (PR) [57] or P4's neuroactive metabolite, allopregnanolone [58,59]. Both mechanisms are capable of increasing NPY expression, as PR is co-localized with NPY in hypothalamic neurons [60] and administration of allopregnanolone induces feeding in rodents [61,62] and increases expression of NPY [63]. We cannot discern from the current study if either or both of these P4 mechanisms are responsible for the weight gain upon P4 treatment.

Alterations in abdominal adiposity were consistent with the changes in body weight, as E2 decreased and addition of P4 to the E2 treatment increased sagittal abdominal circumference. Hormone effects on adiposity are likely mediated through changes in gene expression in adipocytes, as these cells express both E2 and P4 receptors [64–66]. E2 decreased abdominal circumference in all females, regardless of social status, an effect in harmony with the observation that E2 decreases white adipose tissue (WAT) mass in rodents and women [67,68] via activation of ER α [69]. Interestingly, even though subordinate animals did not lose as much weight as dominant animals on E2 treatment, they did lose abdominal adiposity, suggesting that their weight was redistributed on this hormonal condition possibly via increased energy expenditure [70]. There were no social status differences in serum insulin and glucose levels that corresponded with increased body weight and SAC in dominant animals. However, E2 replacement significantly lowered serum insulin without affecting glucose in all females, supporting data that E2 improves insulin sensitivity in a number of species and models [71,72]. In contrast, P4 in combination with E2 increased abdominal circumference and circulating insulin levels in all females, an effect consistent with the fact that P4 increases WAT [73,74].

Our data support previous observations that dominant females had significantly higher serum leptin compared with subordinates [5,40]. This difference was maintained through each of the four different treatment conditions, despite significantly greater weight loss in dominant females during E2 replacement. Knowing E2-induced weight loss occurs independent of leptin [75], we expected that serum leptin would be decreased by the weight loss induced by E2 administration but this hypothesis was not supported. The data suggest that any decrease in leptin resulting from a loss of body fat during E2 treatment was compensated for by a direct effect of E2 increasing leptin synthesis and release. However, data supporting an E2-induced increase in leptin in rodents or in vitro models are equivocal [76–80]. Other approaches also report mixed results, as leptin may vary across the ovulatory cycle but is not necessarily lower during the periovulatory phase in cycling women [81,82], and exogenous E2 administration in postmenopausal women is shown to increase [83,84] and decrease plasma leptin [85]. The weight loss induced by E2 in obese mice is accompanied by reduced leptin gene expression and lower serum levels [78]. However, studies of non-obese, ovariectomized rats [86] or women [87] show that serum leptin levels do not decrease following E2 replacement despite a significant loss of body weight. Thus, it appears that the regulation of leptin synthesis following E2-induced weight loss in obese versus non-obese females may be different, but the mechanisms are undefined.

The effects of social status on body weight, SAC, and the response to E2 administration were not affected by 5HTT. We did however corroborate our previous finding that females with an l/l 5HTT genotype were overall larger than females with s- variant of the gene and that dominant l/l females had higher sagittal adiposity than all other groups of females [5,28]. The overall greater body size associated with the l/l 5HTT genotype is seemingly contradictory to the reports in humans linking the s-variant 5HTT allele to higher rates of

obesity [88,89]. However, our effect of genotype is observed in monkeys maintained on a low-fat diet while humans are likely eating a variety of obesigenic diets. The question of whether 5HTT genotype modifies stress-induced changes in consumption of high caloric diets remains to be addressed.

In conclusion, the results of the present study show that hormonal replacement to ovariectomized female monkeys fed a low-fat, high-fiber diet affects body weight, estimates of body fat, and circulating insulin, and that these effects are modified by the social stress of subordination. Our data show that while E2 decreases body weight in all females, it does so less in females under chronic social stress who are smaller to begin with. It would seem these results are more directly applicable to postmenopausal women or premenopausal women whose ovaries have been removed than premenopausal women. The prevalence of abdominal obesity increases during the peri-menopause and early postmenopausal period [90,91] supports a role of E2 in regulating food intake and body weight [19]. What is unknown is how social stress modulates postmenopausal weight gain in women eating a typical American diet, quite unlike the diet fed the monkeys, and whether this is influenced by E2 replacement. The other question is whether these results are relevant for premenopausal women or monkeys. Chronic exposure to psychosocial stressors in females can often produce anovulation and low but measureable levels of E2 [7,92]. It is unclear how this noncyclic pattern of E2 affects body weight regulation in this population. While E2 does produce cyclic changes in body weight in ovulatory females [93,94], it is unlikely these changes in body weight put females at metabolic risk. Rather, the elevated levels of E2 associated with a decrease in body weight and food intake occur in the context of increased activity and arousal [50], improve peripheral and central glucose utilization [95], and stimulate more prosocial behaviors [96], all functioning to facilitate mate selection and reproduction.

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Figure 1. Mean $\pm$ SEM rates of aggressive behavior received and submissive behavior emitted by females at each social dominance rank. Rates of aggression received and submission emitted were significantly higher ($p < 0.002$) in animals categorized as subordinate females (ranks 3 – 5) compared with those categorized as dominant (ranks 1 and 2).

Figure 2. Mean $\pm$ SEM of changes in body weight (A) and sagittal abdominal circumference (SAC; B) from the beginning to the end of each 4-week hormonal replacement condition. Estradiol significantly attenuated body weight and SAC compared to all other treatments, depicted by an asterisk. Letters in panel (A) highlight the status difference in E2's ability to decrease body weight.

Table 1

Mean $\pm$ SEM for body weight (kg) and sagittal abdominal circumference (cm) collapsed across treatments over the course of the 16-week study as a function of dominance status and 5HTT genotype. Letters indicate significant main effects of status while numbers indicate significant main effects of genotype. Post hoc analyses of the significant status by genotype interaction on sagittal circumference revealed that dominant, l/l females had greater abdominal adiposity than all other groups as indicated by an asterisk.

Status	Body Weight (kg)			Sagittal Abdominal Circumference (cm)		
	5HTT Genotype			5HTT Genotype		
		l/l	s-variant		l/l	s-variant
		8.33 $\pm$ 0.30 ¹	7.37 $\pm$ 0.32 ²		39.50 $\pm$ 1.23 ¹	35.13 $\pm$ 1.21 ²
Dominant	8.35 $\pm$ 0.33 ^a	9.16 $\pm$ 0.46	7.54 $\pm$ 0.46	39.54 $\pm$ 1.33 ^a	43.68 $\pm$ 1.88 ^b	35.33 $\pm$ 1.60
Subordinate	7.35 $\pm$ 0.27 ^b	7.51 $\pm$ 0.40	7.19 $\pm$ 0.38	35.09 $\pm$ 1.12 ^b	35.40 $\pm$ 1.88	34.86 $\pm$ 1.53

Table 2

Metabolic hormone concentrations (mean $\pm$ SEM) at the end of each treatment phase. Dominant females had significantly higher levels of serum leptin compared to subordinates, regardless of treatment ($p = 0.05$). E2 decreased serum insulin concentrations, as indicated by different letters for each of the four treatment conditions.

Metabolic measure	Treatment	Dominant		Subordinate	
		I/I SHHT genotype	s-variant SHHT genotype	I/I SHHT genotype	s-variant SHHT genotype
Leptin (ng/ml)	C	34.88 $\pm$ 4.19	41.03 $\pm$ 4.19	27.67 $\pm$ 3.57	30.98 $\pm$ 3.42
	E2	37.05 $\pm$ 5.79	47.03 $\pm$ 5.79	28.47 $\pm$ 4.94	32.52 $\pm$ 4.73
	P4	41.28 $\pm$ 5.01	43.06 $\pm$ 5.01	29.66 $\pm$ 4.23	32.14 $\pm$ 4.09
	E2 + P4	32.94 $\pm$ 5.33	41.85 $\pm$ 5.33	30.45 $\pm$ 4.55	35.56 $\pm$ 4.35
Insulin (μ U/ml)	C ^b	92.37 $\pm$ 19.76	79.83 $\pm$ 19.76	77.21 $\pm$ 16.85	55.27 $\pm$ 16.13
	E2 ^a	67.17 $\pm$ 14.42	46.27 $\pm$ 14.42	41.08 $\pm$ 12.30	65.20 $\pm$ 11.77
	P4 ^b	124.2 $\pm$ 33.52	79.66 $\pm$ 33.52	87.54 $\pm$ 28.58	109.2 $\pm$ 27.37
	E2 + P4 ^b	125.9 $\pm$ 28.36	60.51 $\pm$ 28.36	66.27 $\pm$ 24.18	88.91 $\pm$ 23.15
Glucose (mg/dl)	C	89.99 $\pm$ 9.81	74.76 $\pm$ 9.81	71.85 $\pm$ 8.36	66.07 $\pm$ 8.01
	E2	78.10 $\pm$ 6.91	71.87 $\pm$ 6.91	63.11 $\pm$ 5.89	65.37 $\pm$ 5.64
	P4	86.81 $\pm$ 8.93	71.11 $\pm$ 8.93	68.80 $\pm$ 7.62	73.73 $\pm$ 7.29
	E2 + P4	82.79 $\pm$ 7.70	68.25 $\pm$ 7.70	70.39 $\pm$ 6.57	64.61 $\pm$ 6.29