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Oestradiol differentially influences feeding behaviour depending on diet composition in female rhesus monkeys

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Abstract

In females, cyclical changes in the ovarian hormone oestradiol are known to modulate feeding behaviour. However, what is less clear is how these behavioural effects of oestradiol are modified by the macronutrient content of a diet. Here we present data showing that oestradiol treatment results in both significantly smaller meals and reduced total caloric intake in ovariectomised, socially housed female rhesus macaques when only chow diet is available. Conversely, during a choice dietary condition where both palatable and chow options are available, oestradiol treatment had no observable, attenuating effect on caloric intake. During this choice dietary phase, all animals consumed more of the palatable diet than chow diet; however, oestradiol treatment appeared to further increase preference for the palatable diet. Finally, oestradiol treatment increased snacking behaviour, i.e. consumption of calories outside of empirically defined meals, regardless of diet condition. These findings illustrate how oestradiol differentially influences feeding behaviour depending on the dietary environment and provides a framework in which we can begin to examine the mechanisms underlying these observed changes.

Introduction

Recent data indicate that 36% of the United States population is obese and thus, has an increased risk for a number of adverse health outcomes (Apovian 2010, Flegal, Carroll et al. 2010). Obesity is clearly a complex disease with multiple genetic (Clement, Vaisse et al. 1998, Farooqi, Keogh et al. 2003, Farooqi and O'Rahilly 2005, Farooqi, Wangenstein et al. 2007) and environmental determinants (Stunkard, Foch et al. 1986, Stunkard, Harris et al. 1990, Maes, Neale et al. 1997), many of which influence satiety and orexigenic signals. Understanding these mechanisms that regulate food choice and appetite are critical to our understanding of the onset and progression of obesity.

Cyclical changes in the ovarian hormone oestradiol are important signals that may influence dietary preferences and consumption patterns in females. Studies in animal models show that increased oestradiol directs motivation towards mating and away from eating when females are fertile (Wade 1972, Wallen and Zehr 2004, Klingerman, Krishnamoorthy et al. 2010). Indeed, cyclical changes in food intake have been observed in many animal species across the estrus cycle, with consistent decreases in caloric intake around the time of ovulation. Within rodent models decreased food intake is observed after the peri-ovulatory

surge in estrogen (Butcher, Collins et al. 1974, Smith, Freeman et al. 1975, Eckel 2011). Additionally, ovariectomy increases daily food intake and weight gain (Mook, Kenney et al. 1972, Wade 1975, Blaustein and Wade 1976, Wade and Gray 1979, Laudenslager, Wilkinson et al. 1980, McElroy and Wade 1987, Varma, Chai et al. 1999), and oestradiol replacement is sufficient to reduce food intake and body weight (Wade 1972, Blaustein and Wade 1976, Butera and Beikirch 1989, Geary and Asarian 1999, Varma, Chai et al. 1999, Eckel, Houtp et al. 2000, Eckel 2004, Asarian and Geary 2006, Santollo, Wiley et al. 2007).

Unlike rodents, humans and other cercopithecine primates have menstrual cycles characterized by a long follicular phase and a prolonged luteal phase following ovulation (Knobil 1980). In these species a peri-ovulatory nadir in food intake of varied diets is often described in both women (Lissner, Stevens et al. 1988, Gong, Garrel et al. 1989, Fong and Kretsch 1993, Johnson, Corrigan et al. 1994, Eck, Bennett et al. 1997) and nonhuman primates (Gilbert and Gillman 1956, Czaja 1978, Rosenblatt, Dyrenfurth et al. 1980). Indeed, the prevailing hypothesis is that the peri-ovulatory rise in oestradiol targets hindbrain and hypothalamic regions to alter processing and feedback signals (Roepke 2009) thereby reducing caloric intake (for review see (Eckel 2004, Eckel 2011)). Specifically, oestradiol's effects are thought to be mediated through multiple biological mechanisms including increasing the satiety potency of cholecystokinin (Geary, Trace et al. 1994, Eckel and Geary 1999, Geary and Asarian 2001, Asarian and Geary 2002), serotonin (Simansky 1996, Eckel 2004), and glucagon (Geary and Asarian 2001), and attenuating the orexigenic potency of ghrelin (Clegg, Brown et al. 2007) and neuropeptide Y (Santollo and Eckel 2008). Through these multiple pathways, oestradiol treatment resulted in reduced meal size and reduced caloric intake in several models (Blaustein and Wade 1976, Laviano, Meguid et al. 1996).

In contrast to the clear cyclic effects of oestradiol upon total energy intake, multiple studies within rodent models have examined oestradiol's effect on dietary selection with conflicting results. While some studies show no effect of ovariectomy upon diet selection (Kanarek and Beck 1980), others have shown that rising oestradiol leads to increased fat consumption (Bartness and Waldbillig 1984, Leibowitz, Akabayashi et al. 1998) and decreased carbohydrate but not protein intake (Wurtman and Baum 1980). In women, some studies have reported reductions in protein and carbohydrate intake coinciding with increased oestrogen levels at ovulation (Lyons, Truswell et al. 1989), while others have reported increased fat intake in the 10 days prior to menstruation (Tarasuk and Beaton 1991). However no consistent effects of oestradiol upon macronutrient selection have emerged (For review see (Dye and Blundell 1997)).

In much the same way, it is unclear how social stress and diet composition interact to influence the effects of oestradiol upon feeding behaviour. In animal models, stressor exposure consistently suppresses appetite when animals are fed a laboratory diet (Krahn, Gosnell et al. 1990, Harris, Zhou et al. 1998, Heinrichs and Richard 1999), and this effect is possibly potentiated by oestradiol in females (Stevens 1989, Kuriyama and Shibasaki 2004). However, a body of data is emerging that shows stress exposure significantly increases caloric consumption when highly palatable foods are available, which is possibly as a result of emotional feeding and may increase risk for obesity (see (Warne 2009)for review).

Recent data suggests that stressor exposure increases consumption of a calorically dense diets in rodents of both sexes (Meisel, Hays et al. 1990, Cottone, Sabino et al. 2009, Foster, Warne et al. 2009, Krolow, Noschang et al. 2010), female rhesus monkeys (Wilson, Fisher et al. 2008, Arce, Michopoulos et al. 2010, Michopoulos, Toufexis et al. 2012) and women (Epel, McEwen et al. 2000, Epel, Lapidus et al. 2001, Tomiyama, Dallman et al. 2011). What is less clear is how oestradiol affects the consumption of calorically dense diets in the context of stressor exposure. On the one hand ovariectomy increases intake of a palatable diet within both binge-resistant and binge-prone rats (Klump, Suisman et al. 2011), suggesting oestradiol in the absence of chronic stress may limit intake of this diet type as well. In contrast, in ovariectomized rats the combination of chronic stress and oestradiol treatment resulted in increased intake of a sweet palatable food when compared to either chronically stressed subjects not receiving oestradiol, or unstressed subjects receiving estradiol, suggesting that the combination of chronic stress and estradiol treatment may actually increase consumption of a palatable diet (Gamaro, Prediger et al. 2003). Therefore, further investigation is required to determine if the effects of oestradiol upon feeding behaviour may be modified by both diet composition and social stress.

When housed socially, female rhesus monkeys organize themselves into a linear dominance hierarchy wherein subordinate members are under constant, unpredictable harassment by more dominant animals (Bernstein and Gordon 1974, Bernstein, Gordon et al. 1974, Shively and Kaplan 1984). Subordinate status is associated with a dysregulation of the limbic – hypothalamic – pituitary – adrenal (LHPA) axis, most consistently shown by diminished glucocorticoid negative feedback (Shively, Laber-Laird et al. 1997, Jarrell, Hoffman et al. 2008, Kaplan, Chen et al. 2010). Social subordination in female macaques produces a number of stress-related phenotypes (Michopoulos, Higgins et al. 2012) and is a well-established model to study the adverse health effects of psychosocial stress on specific phenotypes including reproductive compromise (Flegal, Carroll et al. 2010, Kaplan, Chen et al. 2010), risk for cardiovascular disease (Kaplan, Chen et al. 2009), emotional dysregulation (Shively, Laber-Laird et al. 1997), immune function (Paiardini, Hoffman et al. 2009), and addiction (Morgan, Grant et al. 2002).

The present study used socially housed, ovariectomised adult female rhesus monkeys to determine how social subordination and diet availability interact to influence the effects of oestradiol on feeding behaviour. This study was designed to test the hypothesis that oestradiol would reduce food intake in both dominant and subordinate females maintained on a standard chow diet that is low in fat and high in fiber. Furthermore, it was predicted that when the dietary environment was changed to include the chow diet as well as a more palatable diet with more fat and sugar, oestradiol would attenuate caloric intake in dominant but not subordinate females. Support for these hypotheses would indicate that the mechanisms of stress-induced emotional feeding in females are insensitive to the satiety-induced effects of oestradiol and that hedonic mechanisms that drive food intake can override homeostatic mechanisms of hunger and satiety.

Methods

Subjects

The subjects were 20 adult female rhesus monkeys (*Macaca mulatta*) housed at the Yerkes National Primate Research Center Field Station at Emory University in indoor/outdoor enclosures as described previously (Jarrell, Hoffman et al. 2008). The 20 subjects were housed in four small groups of five females plus a single male. Although the indoor light cycles were 12hr: 12hr, the natural photoperiod prevailed because females had access to outdoor caging. All females had been ovariectomized previously for use in NIH-funded studies assessing the effects of periodic ovarian hormone replacement on a number of outcomes (Michopoulos, Berga et al. 2011, Michopoulos and Wilson 2011, Michopoulos, Higgins et al. 2012). For the present study, the interval from the last replacement with oestradiol was two months. Table 1 lists the animals' characteristics. The Emory University Animal Care and Use Committee approved this protocol 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".

Social Housing of Subjects

The four social groups had been established for at least two years following procedures described previously (Jarrell, Hoffman et al. 2008). Briefly, animals were removed from the middle portion of the dominance hierarchy in the natal breeding groups, ovariectomised, and then sequentially added to one of four groups in a random order. The dominance hierarchy was quickly established and had been stable since group formation. For the present study, dominance ranks were confirmed during 20 minute observations obtained three times each week as well as during daily rounds. Ranks were determined on the basis of dyadic agonistic interactions in which subordination defined as a female emitted an unequivocal submissive gesture to another animal (Bernstein 1976). As reported previously for these females (Michopoulos, Higgins et al. 2012), assessment of glucocorticoid negative feedback using a dexamethasone suppression test showed these subordinate females are less sensitive to dexamethasone suppression compared to dominant females, an indicator of dysregulation of the LHPA axis.

Quantifying Caloric Intake

Food intake data for each subject were recorded 24 h per day, 7 days each week by use of automated feeding devices that were previously validated (Wilson, Fisher et al. 2008). Briefly, each subject had a unique radio-frequency identification (RFID) chip implanted in each wrist. To obtain food, a subject would reach into the feeder allowing the RFID chip to be identified and a single pellet would be dispensed. During each feeding condition, all food was obtained in this way. The validation of the feeding system shows that dominant females do not restrict access by subordinate animals to the feeder and rarely (~ 1% of the time) take a pellet of food that subordinate females obtained (Wilson, Fisher et al. 2008). Additionally, when a female takes a pellet, she consumes it and does not discard it (Wilson, Fisher et al. 2008). Thus, this automated feeding system allows the continuous quantitation of calories consumed by individual monkeys housed in a social group setting.

Diet Conditions

A portion of these data examining the effects of social subordination upon stress related phenotypes, in the absence of oestradiol, were previously published (Michopoulos, Toufexis et al. 2012, Moore, Lowe et al. 2012). The goals of the present study were to determine how oestradiol affects food intake in female rhesus monkeys in two dietary environments and to determine whether stressor exposure modifies these effects of oestradiol. While our previous study examined the intake of a chow diet before and after access to a palatable diet (Michopoulos, Toufexis et al. 2012), the present analysis of the effects of oestradiol were limited to those females whose food intake was quantified initially during a choice dietary condition when both chow and palatable diets were available followed by a chow only condition. While a study design in which diet order is counterbalanced would be valuable, given our previous reports of the effects of a history of consuming a calorically dense diet on subsequent intake of a more prudent low calorie diet (Arce, Michopoulos et al. 2010, Michopoulos, Higgins et al. 2012), this study was designed to model what women might experience when attempting to limit caloric consumption by eliminating intake of highly palatable foods and adhering to a strict low-fat, high-fiber diet. Thus, the first diet condition was a 21-day dietary choice condition where both chow and palatable diet were available ad libitum. The chow diet was a low-fat, high-fiber monkey diet typically fed to the colony (LabDiet, PMI International, #5038), containing 3.45 kcal/gram distributed as: 16% from protein, 12% from fat, and 72% from carbohydrates. Of the total 2.59 kcal/gram of carbohydrates, 2.44 were derived from fiber and 0.15 from sugar. The palatable diet (Lab Diets Typical American Diet (TAD) #5L0R)) contained 3.73 kcal/gram and it was composed of 36% fat, 18% protein, 16.4% sugar carbohydrate and 29.6% fiber-starch carbohydrate. Both diets had similar amounts of cholesterol and the appropriate micronutrients required to maintain the health of the animals. The specific composition in each diet is shown in Table 2. During this choice dietary phase, each diet was offered in one of the two feeders available within each housing enclosure. The specific feeder containing the palatable diet was switched approximately every 4 days to minimize any location effect. Previous studies have shown that the TAD-L0R is consumed in greater quantity by all animals regardless of rank (Michopoulos, Toufexis et al. 2012), therefore it is assumed to be more palatable than the standard chow diet. The second dietary phase was the no choice phase in which chow diet was present in both feeders within each housing enclosure. A 21-day washout period took place between the two 21-day dietary conditions. During this washout period, animals were given ad lib access to the chow only that was not quantified. Body weight of each subject was measured prior to and every 7 days during each of the dietary conditions.

Oestradiol Treatment

The first fourteen days of both the choice and chow only diet conditions served as the no oestradiol phase. On the morning of day fifteen, monkeys were anaesthetised with ketamine hydrochloride and a Silastic capsule (O.D.=0.183 in, I.D.=0.132 in) filled with crystalline 17 β -oestradiol (Sigma-Aldrich, St Louis MO) was implanted subcutaneously between the scapulae as described previously (74). The length of the capsule was adjusted based on a female's body weight. Animals weighing less than 8kg received a 5cm capsule, animals weighing between 8kg and 10kg received a 5.5cm capsule, and animals over 10kgs received

a 6cm capsule. Days 16 through 21 served as the oestradiol treatment phase. No capsules were implanted during the no oestradiol phase, nor were sham surgery performed. The rationale for not counterbalancing the order of no oestradiol versus oestradiol was to eliminate the possible carryover effects of oestradiol on the immediate subsequent no oestradiol condition. Serum was collected from each study subject on days two and five of both the no oestradiol and oestradiol treatment conditions for analysis of oestradiol as previously described using a commercially available radioimmunoassay (Pazol, Kaplan et al. 2004). The assay has a sensitivity of 5 pg/ml using 100 ml of extracted serum, with an intra- and interassay coefficient of variation (CV) of 5.8 and 11.7%, respectively. Sample values of oestradiol were corrected for extraction efficiency, which exceeded 95%. Serum oestradiol levels during the no treatment week were <5 pg/ml while the average concentration during the oestradiol treatment week was 94.4 ± 5.9 for day 2 and 99.8 ± 4.7 pg/ml for day 5, comparable to circulating level of oestradiol during the mid-follicular phase in gonadally intact monkeys (Michopoulos and Wilson 2011). Serum oestradiol did not vary significantly between dominant and subordinate females ($p > 0.05$).

Meal Patterning

Food intake was recorded and meals were empirically defined using methods described previously (Moore, Lowe et al. 2012) and validated using methodology from a previously published report in rats (Zorrilla, Inoue et al. 2005). Briefly, feeding data were preprocessed using BioDAQ software (Research Diets, New Brunswick NJ). A brute force analysis of the average meal duration and average derivative of food consumption were determined for 3,120 meal parameters consisting of 120 time intervals ranging from 60 to 7200 sec increasing by increments of 60 sec for 26 Kcal values ranging from 5.25 to 140 Kcal increasing in increments of 5.25 Kcal. The resulting empirical definition of a meal as a minimum of 70 kcal consumed with a minimum inter-meal interval of 1300 sec produced consistent categorization in the choice and chow only dietary environments. A minimum meal size of 70 kcal with a set minimum inter-meal interval of 1300 seconds shows an appropriate primary distribution during each individual dietary condition and for all dietary phases collapsed. These trends maintain uniformity when feeding frequency is analyzed separately for day and night hours, with night feeding exhibiting an absence of bimodality or snacking (Moore, Lowe et al. 2012). These parameters showed an appropriate primary distribution during each individual dietary condition and for all dietary phases collapsed. Further, a bimodal distribution of the briefest time periods emerged in the collapsed data and in the choice condition among subjects exposed to the choice condition first, indicating that there are different classes of food intake that are characteristically smaller and/or have characteristically briefer pre-meal intervals than are usually seen before meals. Using these parameters, the average number of meals, the average meal size, and the average number of calories consumed outside of these meals (termed “snack calories”) were calculated for each diet condition and oestradiol treatment phase.

Statistical analyses

Data were summarized as mean $\pm$ standard error of the mean (SEM). Following previously described conventions (Michopoulos, Higgins et al. 2012, Michopoulos, Reding et al. 2012) animals ranked 1 and 2 were considered dominant and animals ranked 3 – 5 were classified

as subordinate. Thus, the present analysis included 8 dominant and 12 subordinate females. Multivariate and univariate analysis of variance models with repeated measures evaluated the main and interaction effect of status, diet condition, and oestradiol treatment on total caloric intake as well as the meal parameters including meal size (kcal), meal number, total calories consumed outside of defined meals or snacks (kcal). Effect sizes for univariate comparisons of specific outcomes were reported as Cohen's d and observed power is reported as well. Because we predicted *a priori* that the effects of oestradiol would be different in the chow only and the choice diet conditions, univariate statistics examined these hormone effects within each condition. All statistical tests used SPSS v.20. Statistical tests with $p < 0.05$ were considered significant.

Results

Multivariate analysis of variance indicated a significant effect of diet condition ($F_{4,15}=9.94$, $p<0.001$), oestradiol treatment ($F_{4,15}=8.34$, $p=0.001$), and status ($F_{4,15}=3.66$, $p=0.03$) upon feeding variables. No significant multivariate interactions were observed between diet and status ($F_{4,15}=1.01$, $p=0.43$), oestradiol treatment and status ($F_{4,15}=0.37$, $p=0.82$), oestradiol treatment and diet ($F_{4,15}=1.67$, $p=0.21$), or oestradiol treatment, diet and status ($F_{4,15}=0.75$, $p=0.58$).

Repeated measure univariate analysis of variance showed significant main effects of diet upon total caloric intake, meal size, meal frequency and snack calories (Table 3), as subjects consumed more total calories, larger meals, more meals, and more snack calories during the choice dietary condition compared with the chow only condition. Main effects of oestradiol treatment were observed in snack calories, as subjects consumed significantly more calories outside of defined meals during the oestradiol treatment phase compared with the no oestradiol phase (Table 4, Figure 1). This effect of oestradiol on snack calories was not significantly influenced by diet condition (Figure 1; $F_{1,18} = 3.90$, $p = 0.064$) or status (data not shown; $F_{1,18} = 1.10$, $p = 0.31$). However, no significant main effects of oestradiol upon other meal parameters, independent of diet condition, were observed (Table 4). Main effects of status were observed on total caloric intake as subordinate animals consumed more calories, more meals per day and more snack calories than dominant counterparts; however no status differences was observed with respect to meal size (Table 5). Finally, no significant interactions upon any of the measured feeding variables were observed between diet and status, diet and oestradiol treatment, oestradiol treatment and status, or between the three factors combined.

Finally, means of each feeding variable in both dominant and subordinate subjects during each of the diet conditions is presented in Table 6.

Choice Condition

While there was no significant diet by oestradiol effect, we hypothesized *a priori* that during the dietary choice condition oestradiol would diminish food intake in dominant, but not subordinate subjects. During the choice dietary condition oestradiol treatment failed to significantly influence total daily caloric intake ($F_{1,18} = 0.048$, $p = 0.83$, $d = -0.31$, observed power = 0.06) (Figure 2a), meal size ($F_{1,18} = 0.28$, $p = 0.60$, $d = 0.93$, observed power =

0.80) (Figure 3a) or daily meal number (3.54 ± 0.28 vs. $3.47 \pm .34$, $F_{1, 18} = 0.27$, $p = 0.87$, $d=0.25$, observed power =0.08). Contradicting our hypothesis, there was no significant interaction between oestradiol treatment and social status upon total daily caloric intake ($F_{1, 18} = 0.11$, $p = 0.74$, observed power =0.06) (Figure 1a), meal size ($F_{1, 18} = 0.008$, $p = 0.93$, observed power =0.05) (Figure 3a) or meal number ($F_{1, 18} = 0.68$, $p = 0.42$, observed power = 0.10) (data not shown), suggesting that estradiol has no appetite suppressing effect regardless of rank when both a chow and palatable diet are available. During the choice condition oestradiol treatment reduced total caloric intake to $90.9\% \pm 48.5\%$ of the total caloric intake observed during the no oestradiol phase within dominant subjects, but increased total caloric intake in subordinate females to $146.2\% \pm 144.5\%$; however, the differences was not significant ($F_{1,19} = 1.10$, $p = 0.31$, $d=-0.51$).

Diet preference during the choice condition was influenced by oestradiol treatment as animals consumed $53.7 \pm 3.2\%$ of their calories from the palatable diet during the no treatment phase, but $69.0 \pm 2.4\%$ during the oestradiol treatment phase ($F_{1,18}=15.62$, $p=0.001$, $d=-5.4$) (Figure 4). This effect was not modified by status ($F_{1, 18}= 0.40$, $p= 0.54$) or the interaction of status and oestradiol treatment ($F_{1,18}= 0.02$, $p= 0.96$) (data not shown).

As reported previously (Michopoulos, Toufexis et al. 2012), a significant effect of social status on caloric intake was observed during the choice dietary condition, as subordinate animals consumed more total daily calories (1366.49 ± 161.41 vs. 799.66 ± 197.68 kcal, $F_{1, 18}= 4.93$, $p = 0.039$, $d=3.14$). Furthermore, in the present cohort of females used here, subordinate animals also consumed more meals per day (4.11 ± 0.30 vs. 2.90 ± 0.37 , $F_{1, 18}= 6.83$, $p = 0.022$, $d=3.59$) compared with dominant animals, but there was no difference in meal size (267.74 ± 28.88 kcal vs. 223.85 ± 35.37 kcal, $F_{1, 18}= 0.924$, $p = 0.35$, $d=1.36$).

Chow Only Condition

Our a priori hypothesis was that during the chow only condition oestradiol would suppress food intake both dominant and subordinate females. Our hypothesis was supported, as oestradiol significantly reduced food intake during the chow only condition indicated by fewer total calories ($F_{1, 18} = 7.70$, $p = 0.013$, $d=2.06$, observed power = 0.75) (Figure 2b) and smaller meals ($F_{1, 18}= 15.52$, $p = 0.001$, $d=3.33$, observed power = 0.96) (Figure 3b). There was no significant effect of oestradiol on meal number (3.1 ± 0.3 vs. 2.7 ± 0.4 , $F_{1, 18} = 2.64$, $p = 0.12$, $d=0.99$, observed power = 0.73). Importantly, there was no significant interaction between oestradiol treatment and social status during the chow only condition upon total caloric intake ($F_{1, 18} = 0.24$, $p = 0.63$, observed power = 0.08) (Figure 2b), meal size ($F_{1, 18} = 0.50$, $p = 0.48$, observed power = 0.10) (Figure 3b) or meal number ($F_{1, 18} = 0.02$, $p = 0.89$, observed power = 0.06) (data not shown).

With respect to the effect of social status during this chow only condition, subordinate animals consumed more total calories per day (987.6 ± 125.9 kcal) compared to dominant animals (562.1 ± 154.1 kcal, $F_{1, 18} = 4.57$, $p = 0.046$, $d= 3.02$, observed power = 0.53), but meal size (226.1 ± 18.8 kcal vs. 178.8 ± 23.0 kcal, $F_{1, 18} = 2.53$, $p = 0.13$, $d=2.24$, observed power = 0.33) and meal number (3.4 ± 0.34 vs. 2.33 ± 0.42 , $F_{1, 18} = 4.26$, $p = 0.054$, $d=3.39$, observed power = 0.57) were not significantly different between dominant and subordinate animals. Finally, during the chow only condition, oestradiol treatment reduced caloric intake

to similar amounts within both dominant ($70.5 \pm 41.5\%$) and subordinate females relative to observed total caloric intake during the no oestradiol phase ($77.0 \pm 37.1\%$; $F_{1,19} = 0.10$, $p = 0.72$, $d = 1.5$)

Body Weight Change

Finally, the percent change in body weight from the start of a diet condition for each of the three subsequent weeks is shown in Figure 5. Diet condition significantly influenced this change ($F_{1,18} = 19.60$, $p < 0.001$, $d = -6.0$) such that animals gained weight during the choice condition but lost weight during the no choice condition (Figure 5). Although the percent change during the choice and the chow-only condition appeared to be progressive loss across weeks, the interaction of diet condition and weeks was not significant ($F_{2,18} = 2.06$, $p = 0.14$) suggesting oestradiol during week 3 did not further alter body weight changes. Despite the consumption of significantly more calories by subordinate animals, the change in body weight as a result of diet was not further influenced by social status ($F_{1,18} = 1.02$, $p = 0.83$)

Discussion

The goals of this study were to examine how oestradiol treatment and social stress influenced feeding behaviour, first in a rich dietary environment, when both palatable and chow diets were available, and later in a dietary condition when only chow diet was available. Analysis of these data led to five important conclusions: 1) During the no choice dietary condition, oestradiol treatment reduced meal size and total caloric intake. 2) During a choice dietary condition oestradiol treatment had no observable appetite decreasing effects. 3) During this same choice condition, oestradiol treatment results in all animals consuming a larger percentage of daily calories from HCD than before treatment. 4) Oestradiol treatment resulted in more snacking behaviour, or calories consumed outside of empirically defined meals, during both the choice and no choice condition. 5) The effects of oestradiol upon feeding behaviour were not significantly impacted by social status in either dietary condition. These conclusions do not support our original hypothesis and instead suggest that the effects of oestradiol upon feeding behaviour are primarily modulated by diet composition rather than exposure to the social stress of subordination in female macaques.

It has been widely reported that stress reduces appetite when animals are fed a laboratory chow diet (Krahn, Gosnell et al. 1990, Harris, Zhou et al. 1998, Heinrichs and Richard 1999), yet our data shows that subordinate, stressed monkeys have higher total caloric intake of the chow diet than do dominants, seemingly contradicting this phenomenon established in rodents. However, within this model it has been previously shown that total caloric intake is not significantly different between dominant and subordinate animals consuming chow diet prior to any exposure to a choice condition (Michopoulos, Toufexis et al. 2012). However, following brief exposure to the choice dietary condition during which subordinate animals show significantly greater caloric intake compared to dominant monkeys, subordinate animals continued to consume a greater number of calories compared to dominant animals when they were returned to a chow only condition (Michopoulos, Toufexis et al. 2012). In the present study, only the choice and subsequent chow only condition were used; therefore,

this observation that subordinate animals consume more total calories during the chow only condition was both expected and replicated previous work using this model.

Changes in body weight among study subjects were consistent with changes in the measured feeding parameters as animals gained weight during the choice condition and lost weight during the no choice phase. Importantly, while the effect of diet upon body weight was clearly significant, no direct effect of oestradiol treatment was observed upon body weight in this study. This is in contrast to what has been observed in rodent studies (Blaustein and Wade 1976, Geary and Asarian 1999, Eckel, Houtp et al. 2000, Eckel 2004), as well as within rhesus macaques (Kemnitz, Gibber et al. 1989, Michopoulos and Wilson 2011). However, estrogen-mediated body weight loss observed within rhesus monkey models were seen over four or more weeks' time (Kemnitz, Gibber et al. 1989, Michopoulos and Wilson 2011). It is possible that if oestradiol treatment were extended, we would see a significant effect of oestradiol upon body weight during the chow condition given the observed clear appetite decreasing effect of oestradiol upon food intake during this diet phase.

The mechanisms underlying the absence of appetite suppressing effects of oestradiol during the choice condition are unknown, but several possibilities exist. One possible factor is that the rewarding value of highly palatable food diminishes oestradiol's enhancement of satiety signals. Oestradiol is known to enhance the satiety potency of cholecystokinin (Geary, Trace et al. 1994, Eckel and Geary 1999, Asarian and Geary 2002), serotonin (Simansky 1996) and glucagon (Geary and Asarian 2001, Eckel 2004) and attenuate the orexigenic potency of ghrelin (Clegg, Brown et al. 2007) and neuropeptide Y (Santollo and Eckel 2008). However, consuming a high caloric diet can elicit activation of reward systems, a consequence that may over-ride basic metabolic need. Previous studies in rodents have shown that consumption of a high caloric diet increases levels of dopamine in the nucleus accumbens (Bassareo and Di Chiara 1999), a finding not seen when consuming a chow diet or palatable food devoid of excess calories (Blackburn, Phillips et al. 1986, Bassareo and Di Chiara 1999). Acute oestradiol treatment within rats has been shown to rapidly increase amphetamine-stimulated release of dopamine in the striatum (Becker 1990, Castner, Xiao et al. 1993, Becker and Rudick 1999) and potassium stimulated release of dopamine in the nucleus accumbens (Thompson and Moss 1994). In this same model organism, oestradiol has also been shown to increase striatal dopamine turnover (Di Paolo, Rouillard et al. 1985) and down-regulate the density of dopamine 2 receptor (Bazzett and Becker 1994). Within cynomolgus macaques reduced dopamine 2 receptor availability has been observed during the follicular phase of the menstrual cycle compared to luteal phase (Czoty, Riddick et al. 2009). These physiological changes result in modified reward-seeking behaviour. For instance, female rats have been shown to self-administer cocaine at a higher rate than male counter parts (Lynch and Carroll 1999, Carroll, Morgan et al. 2002), and oestradiol treatment exacerbates this in female (Lynch, Roth et al. 2001), but not male, subjects (Jackson, Robinson et al. 2006). In women, the rewarding effects of cocaine are reported to be higher in the follicular phase compared to luteal phase (Sofuoglu, Dudish-Poulsen et al. 1999, Evans, Haney et al. 2002) and subsequent studies have also shown that that the subject related effects of amphetamine are positively correlated with salivary oestradiol levels (White, Justice et al. 2002). It should be noted that within women, while no clear trends of cycling have been observed upon macronutrient selection (Dye and Blundell 1997), multiple

studies have shown increased preference for sweet, and therefore potentially rewarding, foods during the luteal phase (Bowen and Grunberg 1990, Fong and Kretsch 1993). Therefore, it is possible that in the present study, the combined effect of a high calorie diet and oestradiol treatment on the dopaminergic system blocks estrogen's enhancement of satiety signals so that animals consume more due to the elevated reward value of the food rather than hunger. Further support for this hypothesis is apparent in the fact that all animals, regardless of status consumed a larger percentage of their calories from HCD during the oestradiol treatment phase.

As previously reported, social rank had a significant influence upon feeding behaviour during the choice condition as subordinate animals consumed significantly more total calories and more meals per day compared to their dominant conspecifics during this dietary phase. In part this hyperphagia may also be partially explained by the finding that exposure to chronic psychosocial stress reduces dopamine 2 receptors (D2R) in mesolimbic regions in monkeys (Shively, Grant et al. 1997, Grant, Shively et al. 1998, Shively 1998, Morgan, Grant et al. 2002) resulting in a hypo-dopaminergic condition (Koob 2000). Additionally, simply eating a calorically dense diet reduces D2R in these same regions but only in some animals (Johnson and Kenny 2010). Thus, it could be hypothesized that eating a palatable diet coupled with exposure to psychosocial stressors in subordinate animals may impair DA pathways and thus further inhibit the ability of oestradiol to enhance satiety signals. During the dietary choice condition, oestradiol reduced caloric intake within dominant animals to ~90% of that observed during the no oestradiol treatment phase whereas total caloric intake was increased to ~146% in subordinate females. Given the variance and small sample size this difference between dominant and subordinate females was not significant. This may not continue to be the case, however, with increased sample sizes or under an extended experimental time course. The findings presented here indicate that it would be valuable for future studies within this model to examine the how the dopaminergic system is modulated by the effects of diet, social stress, and oestradiol levels.

Another potential mechanism for the loss of oestradiol's appetite suppressing effects is that consuming a palatable diet high in fats and sugars may disrupt metabolic pathways that are usually responsive to estrogen. Previous data shows that changes in diet composition act quickly to modulate genome-wide expression patterns (Caimari, Oliver et al. 2010), therefore, one possibility is that consumption of a palatable diet disrupts the normal expression levels of multiple molecules involved in satiety signaling, rendering them insensitive to the effects of oestradiol. Indeed, a growing body of evidence suggests that dietary chemicals significantly influence gene expression in three distinct ways. Dietary macronutrients can act as ligands for transcription factors (Dauncey, White et al. 2001, Jacobs and Lewis 2002), alter concentrations of substrates or intermediates of metabolic pathways, or influence signaling pathways (Clarke 1999, Eastwood 2001) (for review see (Kaput and Rodriguez 2004)). While there is no direct evidence to suggest that consumption of a palatable diet in our experiments affects expression of any particular gene, changes in dietary fat, protein and sugar levels have been shown to directly modify expression of estrogen targets such as leptin (Iritani, Sugimoto et al. 2000, Reseland, Haugen et al. 2001), CCK (Liddle, Carter et al. 1988, Hand, Bruen et al. 2010), glucagon (Wang, Cao et al. 2010), ghrelin (Sanchez, Oliver et al. 2004), and NPY (White, He et al. 1994). Furthermore,

recent microarray analysis within mice has shown that over 500 individual genes are differently expressed within gonadal white adipose tissue between intact and OVX mice (Grove, Fried et al. 2010) suggesting that the combination of oestradiol treatment and diet composition have significant, but unexplored effects on genome wide expression levels. The current data do not allow us to distinguish the potential effect, or lack thereof, of expression changes upon feeding behavior. Nonetheless, in these studies we have developed a framework in which we can simultaneously examine the effects of multiple factors upon feeding behaviour of socially housed nonhuman primates and thus begin to test these individual hypotheses by examining dopaminergic signalling and RNA-seq analysis of gene expression within this model.

While the results presented here suggest the appetite suppressing effects of oestradiol are diet-dependent, it is important to point out that the present study has several limitations. First, both dietary phases used here lasted only 21 days each, with oestradiol treatment taking place for only the final 6 days. Thus, it is not possible to accurately assess how feeding parameters might have changed as the high caloric diet became less novel or how longer treatment periods of oestradiol may have influenced feeding behaviour. In addition, these data do not examine how these animals responded to oestradiol treatment before exposure to the choice diet. Given that diet history has been shown to have a significant impact on feeding behaviour within this model, it is possible that oestradiol treatment may have different effects among animals never exposed to high caloric diet options. However, the data discussed here more accurately model people living in modern western societies who have access to both high caloric and low caloric foods. Furthermore, people attempting to lose weight through “dieting” often limit dietary choices to healthier caloric options, which was modeled within the no choice phase of this study.

Also, we have not examined the effect of adding progesterone to estrogen treatment in our studies. This may be particularly important as progestins are known to increase food intake in nonhuman primates (Czaja 1978, Kemnitz, Gibber et al. 1989), women (Pelkman, Chow et al. 2001), and rodents (Wade and Zucker 1970, Kaur and Kulkarni 2002). Indeed our own data show that progesterone attenuates the weight loss effects of oestradiol in females consuming a chow diet (Michopoulos and Wilson 2011). Equally important is the realization that while ovariectomy is advantageous in an experimental setting, an intact individual experiences peak and valleys in estrogen levels and other reproductive hormones, therefore this should be considered when interpreting the results presented here.

In summary, observations from the current study show that oestradiol treatment reduces both meal size and total caloric intake in all animals when only chow is available. In contrast, during a dietary condition where palatable diet is available, treatment has no short-term appetite reducing effects. Furthermore, during this choice diet condition oestradiol treatment actually increases the percentage of daily calories consumed from the palatable diet. None of these effects are further modified by social rank. Finally, regardless of diet condition or rank, oestradiol treatment increases the snacking behaviour of all animals as they consume more calories outside of defined meals. These findings have clear implications for human health. The diet of a typical American includes a range of macronutrients, most typically high in simple sugars and fats, and thus, it is possible that the appetite reducing effects of

oestradiol may be decreased or even lost depending on the macronutrient content of an individual's diet.

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References

- Apovian CM. The causes, prevalence, and treatment of obesity revisited in 2009: what have we learned so far? *Am J Clin Nutr.* 2010; 91(1):277S–279S. [PubMed: 19906802]
- Arce M, Michopoulos V, Shepard KN, Ha QC, Wilson ME. Diet choice, cortisol reactivity, and emotional feeding in socially housed rhesus monkeys. *Physiol Behav.* 2010; 101:446–455. [PubMed: 20670639]
- Asarian L, Geary N. Cyclic estradiol treatment normalizes body weight and restores physiological patterns of spontaneous feeding and sexual receptivity in ovariectomized rats. *Horm Behav.* 2002; 42(4):461–471. [PubMed: 12488112]
- Asarian L, Geary N. Modulation of appetite by gonadal steroid hormones. *Philos Trans R Soc Lond B Biol Sci.* 2006; 361(1471):1251–1263. [PubMed: 16815802]
- Bartness TJ, Waldbillig RJ. Dietary self-selection in intact, ovariectomized, and estradiol-treated female rats. *Behav Neurosci.* 1984; 98(1):125–137. [PubMed: 6538090]
- Bassareo V, Di Chiara G. Differential responsiveness of dopamine transmission to food-stimuli in nucleus accumbens shell/core compartments. *Neuroscience.* 1999; 89(3):637–641. [PubMed: 10199600]
- Bazzett TJ, Becker JB. Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding. *Brain Research.* 1994; 637(1-2):163–172. [PubMed: 8180794]
- Becker JB. Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. *Neurosci Lett.* 1990; 118(2):169–171. [PubMed: 2125712]
- Becker JB, Rudick CN. Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming: a microdialysis study. *Pharmacology, Biochemistry & Behavior.* 1999; 64(1):53–57.
- Bernstein I. Dominance, aggression and reproduction in primate societies. *Journal of Theoretical Biology.* 1976; 60:459–472. [PubMed: 822241]
- Bernstein IS, Gordon TP. The function of aggression in primate societies. *Am Sci.* 1974; 62(3):304–311. [PubMed: 4857115]
- Bernstein IS, Gordon TP, Rose RM. Aggression and social controls in rhesus monkey (*Macaca mulatta*) groups revealed in group formation studies. *Folia Primatol (Basel).* 1974; 21(2):81–107. [PubMed: 4471987]
- Blackburn JR, Phillips AG, Jakubovic A, Fibiger HC. Increased dopamine metabolism in the nucleus accumbens and striatum following consumption of a nutritive meal but not a palatable non-nutritive saccharin solution. *Pharmacol Biochem Behav.* 1986; 25(5):1095–1100. [PubMed: 3786363]
- Blaustein JD, Wade GN. Ovarian influences on the meal patterns of female rats. *Physiol Behav.* 1976; 17(2):201–208. [PubMed: 1033580]
- Bowen DJ, Grunberg NE. Variations in food preference and consumption across the menstrual cycle. *Physiol Behav.* 1990; 47(2):287–291. [PubMed: 2333344]
- Butcher RL, Collins WE, Fugo NW. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17 β throughout the 4-day estrous cycle of the rat. *Endocrinology.* 1974; 94(6):1704–1708. [PubMed: 4857496]

- Butera PC, Beikirch RJ. Central implants of diluted estradiol: independent effects on ingestive and reproductive behaviors of ovariectomized rats. *Brain Res.* 1989; 491(2):266–273. [PubMed: 2765887]
- Caimari A, Oliver P, Keijer J, Palou A. Peripheral Blood Mononuclear Cells as a Model to Study the Response of Energy Homeostasis-Related Genes to Acute Changes in Feeding Conditions. *OmicS-a Journal of Integrative Biology.* 2010; 14(2):129–141. [PubMed: 20235874]
- Carroll ME, Morgan AD, Lynch WJ, Campbell UC, Dess NK. Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: phenotype and sex differences. *Psychopharmacology.* 2002; 161(3):304–313. [PubMed: 12021834]
- Castner SA, Xiao L, Becker JB. Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies. *Brain Res.* 1993; 610(1):127–134. [PubMed: 8518920]
- Clarke SD. Nutrient regulation of gene and protein expression. *Curr Opin Clin Nutr Metab Care.* 1999; 2(4):287–289. [PubMed: 10453307]
- Clegg DJ, Brown LM, Zigman JM, Kemp CJ, Strader AD, Benoit SC, Woods SC, Mangiaracina M, Geary N. Estradiol-dependent decrease in the orexigenic potency of ghrelin in female rats. *Diabetes.* 2007; 56(4):1051–1058. [PubMed: 17251274]
- Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gormelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature.* 1998; 392(6674):398–401. [PubMed: 9537324]
- Cottone P, Sabino V, Steardo L, Zorrilla EP. Consummatory, anxiety-related and metabolic adaptations in female rats with alternating access to preferred food. *Psychoneuroendocrinology.* 2009; 34(1):38–49. [PubMed: 18842344]
- Czaja JA. Ovarian influences on primate food intake: assessment of progesterone actions. *Physiol Behav.* 1978; 21(6):923–928. [PubMed: 552083]
- Czoty PW, Riddick NV, Gage HD, Sandridge M, Nader SH, Garg S, Bounds M, Garg PK, Nader MA. Effect of menstrual cycle phase on dopamine D2 receptor availability in female cynomolgus monkeys. *Neuropsychopharmacology.* 2009; 34(3):548–554. [PubMed: 18256593]
- Dauncey MJ, White P, Burton KA, Katsumata M. Nutrition-hormone receptor-gene interactions: implications for development and disease. *Proceedings of the Nutrition Society.* 2001; 60(1):63–72. [PubMed: 11310425]
- Di Paolo T, Rouillard C, Bedard P. 17 beta-Estradiol at a physiological dose acutely increases dopamine turnover in rat brain. *Eur J Pharmacol.* 1985; 117(2):197–203. [PubMed: 4076343]
- Dye L, Blundell JE. Menstrual cycle and appetite control: implications for weight regulation. *Hum Reprod.* 1997; 12(6):1142–1151. [PubMed: 9221991]
- Eastwood MA. A molecular biological basis for the nutritional and pharmacological benefits of dietary plants. *QJM.* 2001; 94(1):45–48. [PubMed: 11161136]
- Eck LH, Bennett AG, Egan BM, Ray JW, Mitchell CO, Smith MA, Klesges RC. Differences in macronutrient selections in users and nonusers of an oral contraceptive. *Am J Clin Nutr.* 1997; 65(2):419–424. [PubMed: 9022525]
- Eckel LA. Estradiol: a rhythmic, inhibitory, indirect control of meal size. *Physiol Behav.* 2004; 82(1):35–41. [PubMed: 15234587]
- Eckel LA. The ovarian hormone estradiol plays a crucial role in the control of food intake in females. *Physiol Behav.* 2011; 104(4):517–524. [PubMed: 21530561]
- Eckel LA, Geary N. Endogenous cholecystokinin's satiating action increases during estrus in female rats. *Peptides.* 1999; 20(4):451–456. [PubMed: 10458514]
- Eckel LA, Hout TA, Geary N. Spontaneous meal patterns in female rats with and without access to running wheels. *Physiol Behav.* 2000; 70(3-4):397–405. [PubMed: 11006440]
- Epel E, Lapidus R, McEwen B, Brownell K. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. *Psychoneuroendocrinology.* 2001; 26(1):37–49. [PubMed: 11070333]
- Epel ES, McEwen B, Seeman T, Matthews K, Castellazzo G, Brownell KD, Bell J, Ickovics JR. Stress and body shape: stress-induced cortisol secretion is consistently greater among women with central fat. *Psychosom Med.* 2000; 62(5):623–632. [PubMed: 11020091]

- Evans SM, Haney M, Foltin RW. The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. *Psychopharmacology (Berl)*. 2002; 159(4):397–406. [PubMed: 11823892]
- Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med*. 2003; 348(12):1085–1095. [PubMed: 12646665]
- Farooqi IS, O'Rahilly S. Monogenic obesity in humans. *Annu Rev Med*. 2005; 56:443–458. [PubMed: 15660521]
- Farooqi IS, Wangenstein T, Collins S, Kimber W, Matarese G, Keogh JM, Lank E, Bottomley B, Lopez-Fernandez J, Ferraz-Amaro I, Dattani MT, Ercan O, Myhre AG, Retterstol L, Stanhope R, Edge JA, McKenzie S, Lessan N, Ghodsi M, De Rosa V, Perna F, Fontana S, Barroso I, Undlien DE, O'Rahilly S. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med*. 2007; 356(3):237–247. [PubMed: 17229951]
- Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999–2008. *JAMA*. 2010; 303(3):235–241. [PubMed: 20071471]
- Fong AK, Kretsch MJ. Changes in dietary intake, urinary nitrogen, and urinary volume across the menstrual cycle. *Am J Clin Nutr*. 1993; 57(1):43–46. [PubMed: 8416663]
- Foster MT, Warne JP, Ginsberg AB, Horneman HF, Pecoraro NC, Akana SF, Dallman MF. Palatable foods, stress, and energy stores sculpt corticotropin-releasing factor, adrenocorticotropin, and corticosterone concentrations after restraint. *Endocrinology*. 2009; 150(5):2325–2333. [PubMed: 19106219]
- Gamaro GD, Prediger ME, Lopes JB, Dalmaz C. Interaction between estradiol replacement and chronic stress on feeding behavior and on serum leptin. *Pharmacol Biochem Behav*. 2003; 76(2):327–333. [PubMed: 14592685]
- Geary N, Asarian L. Cyclic estradiol treatment normalizes body weight and test meal size in ovariectomized rats. *Physiol Behav*. 1999; 67(1):141–147. [PubMed: 10463640]
- Geary N, Asarian L. Estradiol increases glucagon's satiating potency in ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol*. 2001; 281(4):R1290–1294. [PubMed: 11557638]
- Geary N, Trace D, McEwen B, Smith GP. Cyclic estradiol replacement increases the satiety effect of CCK-8 in ovariectomized rats. *Physiol Behav*. 1994; 56(2):281–289. [PubMed: 7938239]
- Gilbert C, Gillman J. The changing pattern of food intake and appetite during the menstrual cycle of the baboon (*Papio ursinus*) with a consideration of some of the controlling endocrine factors. *S Afr J Med Sci*. 1956; 21(3–4):75–88. [PubMed: 13442720]
- Gong EJ, Garrel D, Calloway DH. Menstrual cycle and voluntary food intake. *Am J Clin Nutr*. 1989; 49(2):252–258. [PubMed: 2916445]
- Grant KA, Shively CA, Nader MA, Ehrenkaufer RL, Line SW, Morton TE, Gage HD, Mach RH. Effect of social status on striatal dopamine D2 receptor binding characteristics in cynomolgus monkeys assessed with positron emission tomography. *Synapse*. 1998; 29(1):80–83. [PubMed: 9552177]
- Grove KL, Fried SK, Greenberg AS, Xiao XQ, Clegg DJ. A microarray analysis of sexual dimorphism of adipose tissues in high-fat-diet-induced obese mice. *Int J Obes (Lond)*. 2010; 34(6):989–1000. [PubMed: 20157318]
- Hand KV, Bruen CM, O'Halloran F, Giblin L, Green BD. Acute and chronic effects of dietary fatty acids on cholecystokinin expression, storage and secretion in enteroendocrine STC-1 cells. *Molecular Nutrition & Food Research*. 2010; 54:S93–S103. [PubMed: 20352619]
- Harris RB, Zhou J, Youngblood BD, Rybkin II, Smagin GN, Ryan DH. Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. *Am J Physiol*. 1998; 275(6 Pt 2):R1928–1938. [PubMed: 9843882]
- Heinrichs SC, Richard D. The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. *Neuropeptides*. 1999; 33(5):350–359. [PubMed: 10657512]
- Iritani N, Sugimoto T, Fukuda H. Gene expressions of leptin, insulin receptors and lipogenic enzymes are coordinately regulated by insulin and dietary fat in rats. *Journal of Nutrition*. 2000; 130(5):1183–1188. [PubMed: 10801916]

- Jackson LR, Robinson TE, Becker JB. Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. *Neuropsychopharmacology*. 2006; 31(1):129–138. [PubMed: 15920500]
- Jacobs MN, Lewis DFV. Steroid hormone receptors and dietary ligands: a selected review. *Proceedings of the Nutrition Society*. 2002; 61(1):105–122. [PubMed: 12002784]
- Jarrell H, Hoffman JB, Kaplan JR, Berga S, Kinkead B, Wilson ME. Polymorphisms in the serotonin reuptake transporter gene modify the consequences of social status on metabolic health in female rhesus monkeys. *Physiol Behav*. 2008; 93(4-5):807–819. [PubMed: 18190935]
- Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci*. 2010; 13(5):635–641. [PubMed: 20348917]
- Johnson WG, Corrigan SA, Lemmon CR, Bergeron KB, Crusco AH. Energy regulation over the menstrual cycle. *Physiol Behav*. 1994; 56(3):523–527. [PubMed: 7972403]
- Kanarek RB, Beck JM. Role of gonadal hormones in diet selection and food utilization in female rats. *Physiol Behav*. 1980; 24(2):381–386. [PubMed: 7375556]
- Kaplan JR, Chen H, Appt SE, Lees CJ, Franke AA, Berga SL, Wilson ME, Manuck SB, Clarkson TB. Impairment of ovarian function and associated health-related abnormalities are attributable to low social status in premenopausal monkeys and not mitigated by a high-isoflavone soy diet. *Human Reproduction*. 2010; 25(12):3083–3094. [PubMed: 20956266]
- Kaplan JR, Chen H, Manuck SB. The relationship between social status and atherosclerosis in male and female monkeys as revealed by meta-analysis. *Am J Primatol*. 2009; 71(9):732–741. [PubMed: 19452517]
- Kaput J, Rodriguez RL. Nutritional genomics: the next frontier in the postgenomic era. *Physiol Genomics*. 2004; 16(2):166–177. [PubMed: 14726599]
- Kaur G, Kulkarni SK. Evidence for serotonergic modulation of progesterone-induced hyperphagia, depression and algesia in female mice. *Brain Res*. 2002; 943(2):206–215. [PubMed: 12101043]
- Kemnitz JW, Gibber JR, Lindsay KA, Eisele SG. Effects of ovarian hormones on eating behaviors, body weight, and glucoregulation in rhesus monkeys. *Horm Behav*. 1989; 23(2):235–250. [PubMed: 2663699]
- Klingerman CM, Krishnamoorthy K, Patel K, Spiro AB, Struby C, Patel A, Schneider JE. Energetic challenges unmask the role of ovarian hormones in orchestrating ingestive and sex behaviors. *Horm Behav*. 2010; 58(4):563–574. [PubMed: 20624393]
- Clump KL, Suisman JL, Culbert KM, Kashy DA, Keel PK, Sisk CL. The effects of ovariectomy on binge eating proneness in adult female rats. *Horm Behav*. 2011; 59(4):585–593. [PubMed: 21376721]
- Knobil E. The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res*. 1980; 36:53–88. [PubMed: 6774388]
- Koob GF. Neurobiology of addiction. Toward the development of new therapies. *Ann N Y Acad Sci*. 2000; 909:170–185. [PubMed: 10911930]
- Krahn DD, Gosnell BA, Majchrzak MJ. The anorectic effects of CRH and restraint stress decrease with repeated exposures. *Biol Psychiatry*. 1990; 27(10):1094–1102. [PubMed: 2340320]
- Krolow R, Noschang CG, Arcego D, Andreazza AC, Peres W, Goncalves CA, Dalmaz C. Consumption of a palatable diet by chronically stressed rats prevents effects on anxiety-like behavior but increases oxidative stress in a sex-specific manner. *Appetite*. 2010; 55(1):108–116. [PubMed: 20347900]
- Kuriyama H, Shibasaki T. Sexual differentiation of the effects of emotional stress on food intake in rats. *Neuroscience*. 2004; 124(2):459–465. [PubMed: 14980395]
- Laudenslager ML, Wilkinson CW, Carlisle HJ, Hammel HT. Energy-Balance in Ovariectomized Rats with and without Estrogen Replacement. *American Journal of Physiology*. 1980; 238(5):R400–R405. [PubMed: 7377378]
- Laviano A, Meguid MM, Gleason JR, Yang ZJ, Renvyle T. Comparison of long-term feeding pattern between male and female Fischer 344 rats: influence of estrous cycle. *Am J Physiol*. 1996; 270(2 Pt 2):R413–419. [PubMed: 8779873]

- Leibowitz SF, Akabayashi A, Alexander JT, Wang J. Gonadal steroids and hypothalamic galanin and neuropeptide Y: role in eating behavior and body weight control in female rats. *Endocrinology*. 1998; 139(4):1771–1780. [PubMed: 9528961]
- Liddle RA, Carter JD, McDonald AR. Dietary-Regulation of Rat Intestinal Cholecystokinin Gene-Expression. *Journal of Clinical Investigation*. 1988; 81(6):2015–2019. [PubMed: 2454954]
- Lissner L, Stevens J, Levitsky DA, Rasmussen KM, Strupp BJ. Variation in energy intake during the menstrual cycle: implications for food-intake research. *Am J Clin Nutr*. 1988; 48(4):956–962. [PubMed: 3421205]
- Lynch WJ, Carroll ME. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. *Psychopharmacology (Berl)*. 1999; 144(1):77–82. [PubMed: 10379627]
- Lynch WJ, Roth ME, Mickelberg JL, Carroll ME. Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. *Pharmacol Biochem Behav*. 2001; 68(4):641–646. [PubMed: 11526960]
- Lyons PM, Truswell AS, Mira M, Vizzard J, Abraham SF. Reduction of food intake in the ovulatory phase of the menstrual cycle. *Am J Clin Nutr*. 1989; 49(6):1164–1168. [PubMed: 2729155]
- Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet*. 1997; 27(4):325–351. [PubMed: 9519560]
- McElroy JF, Wade GN. Short- and long-term effects of ovariectomy on food intake, body weight, carcass composition, and brown adipose tissue in rats. *Physiol Behav*. 1987; 39(3):361–365. [PubMed: 3575476]
- Meisel RL, Hays TC, Del Paine SN, Luttrell VR. Induction of obesity by group housing in female Syrian hamsters. *Physiol Behav*. 1990; 47(5):815–817. [PubMed: 2388935]
- Michopoulos V, Berga SL, Wilson ME. Estradiol and progesterone modify the effects of the serotonin reuptake transporter polymorphism on serotonergic responsivity to citalopram. *Exp Clin Psychopharmacol*. 2011; 19(6):401–408. [PubMed: 21843009]
- Michopoulos V, Higgins M, Toufexis D, Wilson ME. Social subordination produces distinct stress-related phenotypes in female rhesus monkeys. *Psychoneuroendocrinology*. 2012; 37(7):1071–1085. [PubMed: 22244748]
- Michopoulos V, Reding KM, Wilson ME, Toufexis D. Social subordination impairs hypothalamic-pituitary-adrenal function in female rhesus monkeys. *Horm Behav*. 2012; 62(4):389–399. [PubMed: 22940527]
- Michopoulos V, Toufexis D, Wilson ME. Social stress interacts with diet history to promote emotional feeding in females. *Psychoneuroendocrinology*. 2012; 37(9):1479–1490. [PubMed: 22377541]
- Michopoulos V, Wilson ME. Body weight decreases induced by estradiol in female rhesus monkeys are dependent upon social status. *Physiology & Behavior*. 2011; 102(3-4):382–388. [PubMed: 21130792]
- Mook DG, Kenney NJ, Roberts S, Nussbaum AI, Rodier WI 3rd. Ovarian-adrenal interactions in regulation of body weight by female rats. *J Comp Physiol Psychol*. 1972; 81(2):198–211. [PubMed: 5084436]
- Moore CJ, Lowe J, Michopoulos V, Ulam P, Toufexis D, Wilson ME, Johnson ZP. Small changes in meal patterns lead to significant changes in total caloric intake: Effects of diet and social status on food intake in female rhesus monkeys. *Appetite*. 2012 in review.
- Morgan D, Grant KA, Gage HD, Mach RH, Kaplan JR, Prioleau O, Nader SH, Buchheimer N, Ehrenkaufer RL, Nader MA. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nature Neuroscience*. 2002; 5(2):169–174.
- Paiardini M, Hoffman J, Cervasi B, Ortiz AM, Stroud F, Silvestri G, Wilson ME. T-cell phenotypic and functional changes associated with social subordination and gene polymorphisms in the serotonin reuptake transporter in female rhesus monkeys. *Brain Behav Immun*. 2009; 23(2):286–293. [PubMed: 18992804]
- Pazol K, Kaplan JR, Abbott D, Appt SE, Wilson ME. Practical measurement of total and bioavailable estradiol in female macaques. *Clinica chimica acta; international journal of clinical chemistry*. 2004; 340(1-2):117–126.

- Pelkman CL, Chow M, Heinbach RA, Rolls BJ. Short-term effects of a progestational contraceptive drug on food intake, resting energy expenditure, and body weight in young women. *Am J Clin Nutr.* 2001; 73(1):19–26. [PubMed: 11124744]
- Reseland JE, Haugen F, Hollung K, Solvoll K, Halvorsen B, Brude IR, Nenseter MS, Christiansen EN, Drevon CA. Reduction of leptin gene expression by dietary polyunsaturated fatty acids. *J Lipid Res.* 2001; 42(5):743–750. [PubMed: 11352981]
- Roepke TA. Oestrogen modulates hypothalamic control of energy homeostasis through multiple mechanisms. *J Neuroendocrinol.* 2009; 21(2):141–150. [PubMed: 19076267]
- Rosenblatt H, Dyrenfurth I, Ferin M, vande Wiele RL. Food intake and the menstrual cycle in rhesus monkeys. *Physiol Behav.* 1980; 24(3):447–449. [PubMed: 6769135]
- Sanchez J, Oliver P, Palou A, Pico C. The inhibition of gastric ghrelin production by food intake in rats is dependent on the type of macronutrient. *Endocrinology.* 2004; 145(11):5049–5055. [PubMed: 15284203]
- Santollo J, Eckel LA. Estradiol decreases the orexigenic effect of neuropeptide Y, but not agouti-related protein, in ovariectomized rats. *Behav Brain Res.* 2008; 191(2):173–177. [PubMed: 18453005]
- Santollo J, Wiley MD, Eckel LA. Acute activation of ER alpha decreases food intake, meal size, and body weight in ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol.* 2007; 293(6):R2194–2201. [PubMed: 17942491]
- Shively C, Kaplan J. Effects of social factors on adrenal weight and related physiology of *Macaca fascicularis*. *Physiol Behav.* 1984; 33(5):777–782. [PubMed: 6543015]
- Shively CA. Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. *Biological Psychiatry.* 1998; 44(9):882–891. [PubMed: 9807643]
- Shively CA, Grant KA, Ehrenkaufer RL, Mach RH, Nader MA. Social stress, depression, and brain dopamine in female cynomolgus monkeys. *Ann N Y Acad Sci.* 1997; 807:574–577. [PubMed: 9071402]
- Shively CA, Laber-Laird K, Anton RF. Behavior and physiology of social stress and depression in female cynomolgus monkeys. *Biological Psychiatry.* 1997; 41(8):871–882. [PubMed: 9099414]
- Simansky KJ. Serotonergic control of the organization of feeding and satiety. *Behavioural Brain Research.* 1996; 73(1-2):37–42. [PubMed: 8788474]
- Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology.* 1975; 96(1):219–226. [PubMed: 1167352]
- Sofuoglu M, Dudish-Poulsen S, Nelson D, Pentel PR, Hatsukami DK. Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. *Exp Clin Psychopharmacol.* 1999; 7(3):274–283. [PubMed: 10472516]
- Stevens R. Estradiol benzoate potentiates the effects of body-restraint in suppressing food intake and reducing body weight. *Physiol Behav.* 1989; 45(1):1–5. [PubMed: 2727122]
- Stunkard AJ, Foch TT, Hrubec Z. A twin study of human obesity. *JAMA.* 1986; 256(1):51–54. [PubMed: 3712713]
- Stunkard AJ, Harris JR, Pedersen NL, McClearn GE. The Body-Mass Index of Twins Who Have Been Reared Apart. *New England Journal of Medicine.* 1990; 322(21):1483–1487. [PubMed: 2336075]
- Tarasuk V, Beaton GH. Menstrual-cycle patterns in energy and macronutrient intake. *Am J Clin Nutr.* 1991; 53(2):442–447. [PubMed: 1989411]
- Thompson TL, Moss RL. Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. *J Neurochem.* 1994; 62(5):1750–1756. [PubMed: 8158125]
- Tomiya AJ, Dallman MF, Epel ES. Comfort food is comforting to those most stressed: Evidence of the chronic stress response network in high stress women. *Psychoneuroendocrinology.* 2011
- Varma M, Chai JK, Meguid MM, Laviano A, Gleason JR, Yang ZJ, Blaha V. Effect of estradiol and progesterone on daily rhythm in food intake and feeding patterns in Fischer rats. *Physiology & Behavior.* 1999; 68(1-2):99–107. [PubMed: 10627068]

- Wade GN. Gonadal hormones and behavioral regulation of body weight. *Physiol Behav.* 1972; 8(3): 523–534. [PubMed: 4556652]
- Wade GN. Some Effects of Ovarian Hormones on Food-Intake and Body-Weight in Female Rats. *Journal of Comparative and Physiological Psychology.* 1975; 88(1):183–193. [PubMed: 1120795]
- Wade GN, Gray JM. Gonadal Effects on Food-Intake and Adiposity - Metabolic Hypothesis. *Physiology & Behavior.* 1979; 22(3):583–593. [PubMed: 379889]
- Wade GN, Zucker I. Modulation of food intake and locomotor activity in female rats by diencephalic hormone implants. *J Comp Physiol Psychol.* 1970; 72(2):328–336. [PubMed: 5489460]
- Wallen K, Zehr JL. Hormones and history: the evolution and development of primate female sexuality. *J Sex Res.* 2004; 41(1):101–112. [PubMed: 15216429]
- Wang L, Cao HW, Zhang J, Tang CW, Ji QH. Expression of glucagon in gestational and diet-induced hyperlipidemic gestational rats. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi.* 2010; 26(4):359–362. [PubMed: 20368116]
- Warne JP. Shaping the stress response: interplay of palatable food choices, glucocorticoids, insulin and abdominal obesity. *Mol Cell Endocrinol.* 2009; 300(1-2):137–146. [PubMed: 18984030]
- White BD, He B, Dean RG, Martin RJ. Low-Protein Diets Increase Neuropeptide-Y Gene-Expression in the Basomedial Hypothalamus of Rats. *Journal of Nutrition.* 1994; 124(8):1152–1160. [PubMed: 8064364]
- White TL, Justice AJ, de Wit H. Differential subjective effects of D-amphetamine by gender, hormone levels and menstrual cycle phase. *Pharmacol Biochem Behav.* 2002; 73(4):729–741. [PubMed: 12213517]
- Wilson ME, Fisher J, Fischer A, Lee V, Harris RB, Bartness TJ. Quantifying food intake in socially housed monkeys: social status effects on caloric consumption. *Physiology & Behavior.* 2008; 94:586–594. [PubMed: 18486158]
- Wurtman JJ, Baum MJ. Estrogen reduces total food and carbohydrate intake, but not protein intake, in female rats. *Physiol Behav.* 1980; 24(5):823–827. [PubMed: 7190713]
- Zorrilla EP, Inoue K, Fekete EM, Tabarin A, Valdez GR, Koob GF. Measuring meals: structure of prandial food and water intake of rats. *Am J Physiol Regul Integr Comp Physiol.* 2005; 288(6):R1450–1467. [PubMed: 15637168]

Figure 1.

Mean $\pm$ SEM average daily caloric intake outside of defined meals (“snacks”) across both diet conditions during the no treatment and oestradiol treatment phases. Snacks consumed during the choice diet condition are shown with open bars while the chow condition is shown with closed bars.

Figure 2.

a: Mean $\pm$ SEM average total daily caloric intake (both meals and snacks) for the choice condition during the no oestradiol and oestradiol treatment phases. Data are shown for dominant (open bars) and subordinate females (closed bars). b: Mean $\pm$ SEM average total daily caloric intake (both meals and snacks) for the chow only condition during the no oestradiol and oestradiol treatment phases. Data are shown for dominant (open bars) and subordinate females (closed bars).

Figure 3.

a: Mean $\pm$ SEM average meal size for the choice condition during the no oestradiol and oestradiol treatment phases. Data are shown for dominant (open bars) and subordinate females (closed bars). b: Mean $\pm$ SEM average meal size for the chow only condition during the no oestradiol and oestradiol treatment phases. Data are shown for dominant (open bars) and subordinate females (closed bars).

Figure 4. Percent of total caloric intake consisting of each diet during the choice dietary trial. Closed bars show the percentage of palatable diet consumed, while open bar show percent chow consumed.

Figure 5. Mean $\pm$ SEM percent change in body weight during each of the three weeks during each diet condition.

Table 1

List of subjects included in analysis with dates of ovariectomy, last oestradiol treatment, date of birth, weight, rank and social group listed.

Animal ID	Birthdate	Date of OVX	Interval from Ovariectomy to Start of Study (years)	Social group	Rank	Weight at Start of Study (kg)	Time from last E2 treatment (months)
RBm4	6/9/1993	12/22/2005	3.8	1	1	9.73	7.1
ROh4	5/5/1993	10/4/2005	4.0	1	2	12.55	7.1
RHn6	4/19/1998	12/2/2005	3.8	1	3	7.09	7.1
RMg5	4/28/1995	8/16/2000	9.1	1	4	7.69	7.1
RVi4	5/10/1993	10/18/2005	3.9	1	5	8.89	6.2
RBe5	4/10/1995	1/6/2006	3.7	2	1	9.69	6.2
RHe4	3/20/1993	10/13/2005	3.9	2	2	10.50	6.2
RMg3	4/13/1991	5/14/2001	8.4	2	3	7.85	6.2
RRb7	4/29/1999	10/4/2005	4.0	2	4	6.43	6.2
RZt5	5/20/1996	11/30/2005	3.8	2	5	7.88	6.2
RZp6	5/9/1998	1/31/2006	3.6	3	1	11.16	7.6
RIz6	4/16/1999	12/1/2005	3.8	3	2	8.28	7.6
RYn5	3/19/1996	11/2/2005	3.9	3	3	7.43	7.6
RRu6	3/9/1999	1/5/2006	3.7	3	4	5.95	7.6
RZd7	1/15/1999	1/23/2006	3.7	3	5	6.20	7.6
RWu4	4/30/1994	1/13/2006	3.7	4	1	7.89	6.8
RIu7	5/9/1999	1/20/2006	3.7	4	2	6.78	6.8
RCv6	3/20/1999	10/7/2005	4.0	4	3	7.17	6.8
RDv6	3/23/1999	11/15/2005	3.9	4	4	7.34	6.8
RVz6	4/19/1999	9/8/2005	4.0	4	5	6.36	6.8

Table 2

Descriptions of Chow and Typical American Diets prepared by Lab Diet, PMI Nutrition International. Each diet has comparable mineral and vitamins

Composition	Chow, Monkey Diet (5037/5038)	Typical American –Soy Primate Diet (5L0R)
Protein	15.7%	17.0%
Fat (ether extract)	5%	15.0%
Fat (acid hydrolysis)	6.0%	15.4%
Cholesterol	75 ppm	635 ppm
Linoleic Acid	1.66%	2.70%
Linolenic Acid	0.1%	0.14%
Arachidonic Acid	< .01%	0.05%
Omega-3 fatty Acids	0.13%	0.26%
Total Saturated Fatty Acids	1.54%	5.30%
Total Monosaturated Fatty Acids	1.68%	6.10%
Fiber	4.5%	8.70%
Nitrogen free extract	59.3%	42.4%
Starch	42.4%	28.4%
Glucose	0.29%	0.04%
Fructose	0.32%	5.54%
Sucrose	2.24%	9.56%
Lactose	1.68%	0%
Gross energy	4.05 kcal/g	4.76 kcal/g
Physiological fuel value	3.45 kcal/g	3.73 kcal/g
Calories provided		
Protein	18.19%	18.29%
Fat	13.09%	36.21%
Carbohydrate	68.72%	45.51%

Table 3

Main effects of diet upon measured feeding variables. Data are presented as means $\pm$ SEM for each feeding variable

Effects of Diet				
Feeding Variable	Choice Condition	No Choice Condition	Statistical Comparison	Cohen's Effect Size
Total Caloric Intake	1083.1 $\pm$ 127.6	774.8 $\pm$ 99.5	$F_{1,18} = 23.52, p < 0.001$	d= 2.69
Meal Size	245.8 $\pm$ 22.8	202.4 $\pm$ 14.9	$F_{1,18} = 5.53, p = 0.03$	d=2.25
Meals Per Day	3.5 $\pm$ 0.2	2.9 $\pm$ 0.3	$F_{1,18} = 7.27, p = 0.015$	d=2.35
Snack Calories	138.6 $\pm$ 8.2	116.3 $\pm$ 5.6	$F_{1,18} = 8.78, p = 0.008$	d=3.27

Table 4

Main effects of Oestradiol treatment upon measured feeding variables. Data are presented as means $\pm$ SEM for each feeding variable

Effect of Oestradiol				
Feeding Variable	No treatment	Oestradiol Treatment	Statistical Comparison	Cohen's Effect Size
Total Caloric Intake	970.2 $\pm$ 98.9	887.7 $\pm$ 159.4	$F_{1, 18} = 0.31, p = 0.59$	d= 0.62
Meal Size	242.0 $\pm$ 15.6	206.1 $\pm$ 23.4	$F_{1, 18} = 2.94, p = 0.10$	d= 1.81
Meals Per Day	3.3 $\pm$ 0.2	3.1 $\pm$ 0.3	$F_{1, 18} = 0.99, p = 0.33$	d=0.78
Snack Calories	116.8 $\pm$ 5.9	138.1 $\pm$ 8.2	$F_{1, 18} = 6.93, p = 0.017$	d= -2.98

Table 5

Main effects of status upon measured feeding variables. Data are presented as means $\pm$ SEM for each feeding variable

Effects of Status				
Feeding Variable	Dominant	Subordinate	Statistical Comparison	Cohen's Effect Size
Total Caloric Intake	680.9 $\pm$ 170.3	1177.0 $\pm$ 139.0	F_{1, 18} = 5.09, p=0.037	d= -3.19
Meal Size	201.3 $\pm$ 26.2	246.9 $\pm$ 21.4	F _{1, 18} =1.82, p=0.20	d= -1.90
Meals Per Day	2.6 $\pm$ 0.4	3.8 $\pm$ 0.3	F_{1, 18} =6.40, p=0.021	d= -3.39
Snack Calories	104.8 $\pm$ 9.1	150.2 $\pm$ 7.5	F_{1, 18} = 14.77, p=0.001	d= -5.44

Table 6

Data are presented as means $\pm$ SEM for each feeding variable within both dominant and subordinate subjects during each of the diet conditions

Chow only Condition				
	Total Caloric intake	Meal size	Meal Number	Snack Calories
Dominant - Control	652.7 $\pm$ 152.0	201.3 $\pm$ 26.6	2.6 $\pm$ 0.4	97.4 $\pm$ 8.1
Dominant - Oestradiol	471.4 $\pm$ 178.9	156.2 $\pm$ 24.3	2.1 $\pm$ 0.6	89.0 $\pm$ 12.4
Subordinate- Control	1117 $\pm$ 124.1	258.4 $\pm$ 21.7	3.7 $\pm$ 0.3	125.2 $\pm$ 6.6
Subordinate- Oestradiol	857.6 $\pm$ 146.0	193.6 $\pm$ 19.8	3.2 $\pm$ 0.4	153.7 $\pm$ 10.1
Choice Diet Condition				
	Total Caloric intake	Meal size	Meal Number	Snack Calories
Dominant - Control	814.4 $\pm$ 211.6	233.7 $\pm$ 31.6	3.1 $\pm$ 0.4	99.4 $\pm$ 12.7
Dominant - Oestradiol	784.9 $\pm$ 333.1	214.0 $\pm$ 52.3	2.7 $\pm$ 0.5	133.4 $\pm$ 18.0
Subordinate- Control	1296.1 $\pm$ 172.8	274.8 $\pm$ 25.7	4.0 $\pm$ 0.4	145.4 $\pm$ 10.4
Subordinate- Oestradiol	1436.9 $\pm$ 272.0	260.7 $\pm$ 42.7	4.2 $\pm$ 0.4	176.4 $\pm$ 14.7