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Puberty Onset in Female Rats: Relation to Fat Intake, Ovarian Steroids and the Peptides, Galanin and Enkephalin, in the Paraventricular and Medial Preoptic Nuclei

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Abstract

Puberty is a time of rapid change, including a marked increase in fat consumption and body fat accrual, particularly in females. The mechanisms underlying these changes are unknown. Building on results obtained in adult rats, the present study in pubertal rats focused on the orexigenic peptides, galanin (GAL) and enkephalin (ENK), in the paraventricular nucleus (PVN) and medial preoptic nucleus (MPN), which are known to be responsive to female steroids and have a role in both energy balance and reproductive function. This study examined female rats maintained on pure macronutrient diets from before weaning (day 15) to day 70. After an initial burst in protein intake (days 21-35), the rats showed an increase specifically in preference for fat, from 15% to 30%. In rats examined at different ages before (day 30) and after (days 45 and 60) puberty, this rise in fat intake was associated with a marked increase, from days 30 to 45, in levels of oestradiol and progesterone and in GAL and ENK mRNA or peptide levels specifically in the PVN and MPN, but not other hypothalamic areas examined. This positive relationship with increased fat intake, steroids and peptides across ages was also observed when comparing pubertal rats that naturally preferred fat (>25% of total diet) to those consuming little fat (<15%) or rats that reached puberty at an early age (days 30-34) to those that were late (days 37-40). These rats with early puberty onset exhibited a strong fat preference 3-4 days before vaginal opening, which was positively related to steroid levels, GAL, fat intake and body fat accrual after puberty. These findings suggest that, in addition to providing a signal for puberty onset, early fat ingestion acting through mechanisms involving the steroids and orexigenic peptides may be related to long-term patterns of eating and body weight regulation.

Keywords

dietary fat; oestradiol; progesterone; hypothalamus; galanin; enkephalin; food intake

Introduction

Puberty is a time of dramatic and sudden change in energy balance as well as reproductive function, particularly in females. There is a marked increase in secretion of gonadotrophin releasing-hormones and the steroid, oestradiol (E_2) (1), and also in preference for dietary fat and body fat accrual (2,3). Studies suggest that increased nutrition before puberty as well as elevated weight gain and body fat may affect reproductive maturation and trigger the early onset of puberty (4,5). These parameters in prepubertal females may also be risk factors for obesity and diabetes later in life (4,6). The brain mechanisms involved in these relationships,

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between early nutrition, reproductive processes and long-term body weight regulation, remain to be characterized.

The tight link known to exist between energy homeostasis and reproduction (7) leads us to focus on brain peptides and brain areas that control both functions. Two such candidates are the orexigenic peptides, galanin (GAL) and enkephalin (ENK), which are heavily expressed in two nuclei, the paraventricular nucleus (PVN) and medial preoptic nucleus (MPN) immediately rostral to the PVN, that project to the external zone of the median eminence (ME) (8-10). These peptides modulate hormone secretion from the anterior pituitary (AP) (11-13) and also have effects on mating behavior (13-16). With puberty characterized by a marked increase in preference for a fat-rich diet and body fat accrual (2,3,17), a specific role for GAL and ENK in pubertal animals is suggested by evidence that these peptides preferentially stimulate ingestion of a fat-rich diet, which enhances fat deposition, and that their receptor antagonists produce the opposite (18-23). Moreover, consumption of a fat-rich diet or injection of a lipid emulsion further stimulates these peptides, suggesting that they function within a positive feedback loop that promotes further ingestion of fat (24-26). This effect of dietary fat on orexigenic peptides is site specific, occurring in the PVN and MPN as well as ME of female rats, although not in the MPN of male rats, and absent in other areas of the hypothalamus or medial preoptic area (MPOA). This evidence supports a role for these two peptides and nuclei in coordinating functions related to feeding and reproduction in female rats, possibly around puberty.

This hypothesis is further supported by evidence linking GAL and ENK in the PVN and MPN to the female steroids, E_2 and progesterone (P4), which like the peptides are found to be affected by the consumption of a fat-rich diet. Neurons expressing GAL and ENK in these nuclei contain the steroid receptors or concentrate E_2 (8,27-30), and the expression of these peptides in the PVN and MPN is stimulated by administration of the steroids (31-35). Further, females show elevated peptide expression and fat preference when compared to males (2,8,32,36,37), and these measures peak during the proestrous phase of the female cycle when E_2 and P4 levels are elevated (36,38,39). In addition to stimulating GAL peptide, these steroids modulate GAL-R1 receptors, which are dense in the PVN and MPN and possibly mediate GAL's feeding-stimulatory effect (40,41). They also have effects of their own on feeding behavior (7), with P4 in E_2-primed rats preferentially stimulating intake of a fat-rich diet (42,43), and they are increased in the circulation by ingestion of a high-fat diet (36,42,44). A role for these steroids in the positive relationship between dietary fat and orexigenic peptides in females is underscored by the finding that ovariectomy greatly attenuates the stimulatory effect of a fat-rich diet on GAL (42). Together, these findings support the possibility that the peptide-expressing neurons in the PVN and MPN, which project to the ME and are responsive to both fat and steroids, may be involved in functions of energy homeostasis closely related to reproduction, particularly around puberty when increased consumption of a fat-rich diet and body fat accrual are essential for reproduction.

To test this idea, measurements were taken of feeding patterns and nutrient preferences around puberty, in addition to circulating steroids and orexigenic peptides. Rats were raised since before weaning on pure macronutrient diets and thus allowed to express their natural preference for fat. Measurements of fat intake, steroid levels, and peptides were recorded and related at different ages before and after puberty. They were also examined in individual animals that differed in their preference for fat or precise age of puberty onset as determined by day of vaginal opening (VO). The results of these experiments, focusing on GAL and ENK in the PVN and MPN, consistently demonstrate a close, positive relationship between these measures in pubertal animals and reveal exaggerated patterns in rats that naturally prefer fat or reach puberty at an early age

Materials and methods

Animals

Time-pregnant, Sprague-Dawley rats (210-240g) from Charles River Breeding Laboratories (Hartford, CT) were delivered to the animal facility on embryonic day 5 (E5). The dams were individually housed in plastic cages and maintained on standard lab chow, in a fully accredited AAALAC facility (22°C, with a 12:12-h light-dark cycle with lights off at 2 pm), according to institutionally approved protocols as specified in the NIH Guide to the Use and Care of Animals and also with approval of the Rockefeller University Animal Care Committee. At birth, the litters were culled to 10 each by removing primarily males, with the 24 h period after birth designated as day 1. The dams and offspring at 15 days of age (day 15) were given *ad libitum* access to 3 pure macronutrient diets (see below), in addition to lab chow. The pups were then weaned on day 21, individually housed, and maintained on the pure macronutrient diets without chow. Over the course of the experiments, nutrient intake and body weight were measured 2-3 times per week, except in Experiment 5 when daily measurements were taken during the period before VO, which ranged from days 30-40.

Diets

The 3 pure macronutrients, fat, carbohydrate and protein, were presented simultaneously in 3 separate jars, as described (45). With macronutrient composition calculated as percent of total kcal, the protein diet (3.7 kcal/g) consisted of 93% casein (Bioserv) mixed with 4% minerals (USP XIV Salt Mixture Briggs, I.C.N. Pharmaceuticals), 2.97% vitamins (Vitamin Diet Fortification Mixture, I.C.N. Pharmaceuticals), and 0.03% cysteine (L-cysteine hydrochloride, I.C.N. Pharmaceuticals). The carbohydrate diet (3.7 kcal/g) was composed of 28% dextrin, 28% cornstarch (I.C.N. Pharmaceuticals) and 37% sucrose (Domino) mixed with 4% minerals and 3% vitamins, while the fat diet (7.7 kcal/g) consisted of 66% lard (Armour) and 20% corn oil (Mazola) mixed with 8% minerals and 6% vitamins.

Test procedures

Five experiments were conducted. The ages examined in these experiments, including days 30, 45, 60 and 70, were chosen based on our previously published study (2), which showed a rise in fat intake from before puberty (day 30) to after puberty (day 45) and a peak in fat intake 2-3 weeks later (days 60-70). In the analyses of steroids and brain peptides as they relate to patterns of macronutrient intake, the post-pubertal rats were sacrificed randomly across the 4 stages of the estrous cycle. While these parameters may show changes across these different stages (36), a recent study of adult rats (42) demonstrates that their close, positive relationship can still be seen at a single stage and independently of sacrifice time, leading us to follow the same protocol in the present study of examining the pubertal rats randomly across the cycle. They were sacrificed by rapid decapitation, after which their brains were removed, trunk blood was collected, and fat pads were dissected.

Experiment 1—Female rats (n=32) raised on the macronutrient diets from 15 days of age (day 15) to day 70 were examined with frequent measurements after weaning (day 21) of body weight, body weight gain, total caloric intake, intake of the pure macronutrients, and preference for these macronutrients as determined by % of total daily intake.

Experiment 2—Female rats (n=42) were similarly raised on the 3 macronutrient diets but were sacrificed at 3 different ages (n=14/age), before puberty (day 30), shortly after puberty (day 45) and at maturity (day 60). Body weight, total caloric intake, and intake of the pure macronutrients were monitored in the same manner as Experiment 1. At the time of sacrifice, unilateral body fat from 3 regions (gonadal, retroperitoneal and inguinal tissue) as well as the mesenteric fat pad were dissected and weighed, with the body fat measure reflecting the sum

of the individual fat pads. Trunk blood was collected for analysis of circulating levels of the hormones, E_2 and P4 as well as insulin and leptin, and the brains were dissected for measurements of peptide levels via radioimmunoassay (RIA), as described below.

Experiment 3—Similar to Experiment 2, female rats (n=30) were raised on the 3 macronutrient diets with frequent measurements taken of body weight and nutrient intake, and they were sacrificed on days 30, 45 and 60 (n=10/age) by rapid decapitation. After sacrifice, the brains were dissected for analysis of peptide gene expression using real-time quantitative PCR (see below).

Experiment 4—An additional set of female rats (n=24) was similarly raised on the 3 macronutrient diets but were sacrificed at a single age, on day 50. These rats were subgrouped (n=8/group) based on their average fat preference from days 45-50, designated "high-fat eaters" consuming >25% fat or "low-fat eaters" consuming <15% fat. Trunk blood was collected for analysis of circulating steroid levels, while the brains were removed for measurement of peptide expression using *in situ* hybridization (see below).

Experiment 5—Similar to Experiments 1, female rats (n=24) were raised on the 3 macronutrient diets from day 15 to day 70, with frequent measurements taken of body weight and nutrient intake. To determine the precise days of puberty onset, the rats were examined daily for VO, from days 29-40. They were subgrouped (n=8/group) based on the day of VO, designed "early VO" if it occurred between days 30 and 34 or "late VO" if it occurred between days 37 and 40. To further characterize these groups, they were sacrificed on day 70, trunk blood was collected for analysis of circulating hormones, and brains were removed for measurements of peptide gene expression using *in situ* hybridization (see below).

Hormone Assays

Both serum 17β-oestradiol (E_2) and progesterone (P4) were assayed using commercially available radioimmunoassay kits from MP Biomedical (Costa Mesa, CA). Serum levels of insulin and leptin were assayed using RIA kits from Linco Research Inc, MO. The hormone assays were performed at different times for different experiments. The sensitivity of the assays is as follows: 7.2 pg/ml for E_2, 0.03 ng/ml for P4, 0.1 ng/ml for insulin, and 0.5 ng/ml for leptin. The intra- and the inter-assay coefficients of variation (%) are: 7.2 and 9.0 for E_2; 6.9 and 8.0 for P4, 4.1 and 3.0 for leptin, and 4.3 and 8.5 for insulin.

Brain areas examined

Nine brain areas, each with a dense concentration of GAL and ENK neurons, were examined using RIA, real-time quantitative PCR, and *in situ* hybridization, as previously described (26,42). For measurements of peptide mRNA and peptide levels in dissected tissue, the rats were sacrificed by rapid decapitation, their brains were immediately placed in a matrix with the ventral surface facing up, and three 1.0 mm coronal sections were made, with the anterior border of the optic chiasm as the anterior boundary. The sections were then placed on a glass slide and the following areas rapidly microdissected bilaterally under a microscope, using the anterior commissure, fornix and third ventricle as landmarks. With coordinates according to the atlas of Paxinos and Watson (46), these areas at 4 anterior-posterior levels were: 1) medial preoptic nucleus (MPN) in the caudal region of the MPOA, at the level of and posterior to the anterior commissure and <0.5 mm immediately anterior to the PVN (Bregma -0.4 to -0.92 mm); 2) suprachiasmatic nucleus (SCN) (Bregma -0.92 to -1.4mm; 3) paraventricular nucleus (PVN) and supraoptic nucleus (SON) (Bregma -0.92 to -2.1 mm); and 4) arcuate nucleus (ARC), ventromedial hypothalamus (VMH), dorsomedial nucleus (DMN), lateral hypothalamus (LH) and median eminence (ME) (Bregma -2.56 to -2.8 mm). These dissections were immediately frozen in liquid nitrogen and stored at -80° C until processed. For analysis

of peptide mRNA using *in situ* hybridization, the brains were rapidly removed and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4° C for 3 days and cryoprotected in 25% sucrose-phosphate buffer at 4° C for a further 72-96 h. They were then frozen at -80° C until day of use.

Radioimmunoassay (RIA)

The dissected tissue was homogenized in 1 mL of 0.1 M acetic acid and centrifuged at 14,000 × *g* for 15 min at 4° C. All of the supernatant was removed, boiled for 10 min and frozen at -80° C until use. Levels of GAL in different brain areas were measured as described elsewhere (36,47).

Real-time quantitative PCR analysis

Real-time quantitative PCR was used to measure GAL and ENK mRNA levels in the PVN and MPN, as described (48). Briefly, total RNA from n=10-14 microdissected samples from each age or diet group was pooled and extracted with Trizol reagent, and cDNA was synthesized with oligo-dT primer. Real-time quantitative PCR was run with SYBR Green core reagents kit (ABI, CA) and *β*-actin as an endogenous control. The levels of target gene expression were quantified relative to the level of *β*-actin by standard curve method. Each study consisted of 4-6 independent runs of PCR in triplicate, and each run included a standard curve, non-template control, and negative RT control. In addition, the specificity of the quantitative PCR was verified with an anatomical negative control by using the corpus callossum in the same brain. No signals above threshold of the two targeted genes were detected by quantitative PCR in all of the controls.

In situ hybridization histochemistry

As previously described (48), digoxigenin-labeled antisense RNA probes and 30 μm free-floating cryostat sections were used for *in situ* hybridization histochemistry. AP-conjugated sheep anti-digoxigenin Fab fragments (1:1000, Roche) and NBT/BCIP (Roche) were used to visualize the signal. Gene expression level was measured by semiquantification with Image-Pro Plus software (Version 4.5, Media Cybernetics Inc., Silver Spring, MD), as reported (42), and was expressed as cells/mm^2, reflecting density of mRNA containing cells. In all analyses, the cell number was counted only on one plane in each section, and only those cells containing a nucleus in the plane (>10 μm^2) were counted, thereby excluding fractions of cells. The average cell density in each nucleus or area for the different groups was compared and statistically analyzed, with the analyses performed by an observer blind to the identity of the animals.

Data Analysis

All values are expressed as mean ± SEM. With a standard statistical package (SPSS), statistical analyses comparing the different measures (food intake, body weight, hormone levels and peptides) for the subgroups were performed using a one-way ANOVA, followed by a Bonferroni post-hoc test for multiple comparisons between groups, or an unpaired Student's *t*-test when appropriate. Within-group measures of macronutrient intake, hormones and peptides were related using a Pearson's product moment correlation. The criterion for use of the term “significant” in the text is that the probability value for a given test is $p<0.05$.

Results

These experiments examined female rats before and after puberty, to determine whether their natural preference for fat and associated body fat accrual were related to changes in circulating hormones and orexigenic peptides in specific brain areas.

Experiment 1: Age-related changes in macronutrient intake

Female rats (n=32) raised on the 3 pure macronutrient diets showed a steady increase with age in their total daily caloric intake until 56 days of age, when it stabilized at approximately 80 kcal (Fig. 1). During the first two weeks after weaning (days 21-35), protein intake increased markedly from 9 to 33 kcal/day, with preference for protein (% of total diet) peaking at 47% on day 35 ($p<0.01$). After day 35, fat intake showed a significant rise, from 7 to 22 kcal/day, with preference for this macronutrient increasing from 17% to 30% by 70 days of age ($p<0.01$). Body weight rose steadily with age (Fig. 1). Before puberty (days 25-35), weight gain was positively correlated with the ingestion of protein ($r=+0.58$, $p<0.01$) but not fat or carbohydrate, while after puberty it was positively related to intake specifically of fat ($r=+0.67$, $p<0.01$). These results reveal two dynamic changes in nutrient preferences across age, a rise in protein preference after weaning followed by a rise in fat preference after day 35.

Experiment 2: Age-related changes in hormones and GAL in relation to macronutrient intake

To investigate the relationship of these eating patterns to changes in circulating hormones and the orexigenic peptide GAL, a second set of female rats (n=42) similarly raised on macronutrient diets was sacrificed and examined at one of 3 ages (n=14/age), either before puberty (day 30), shortly after puberty (day 45), or at maturity (day 60). As in Experiment 1, these rats exhibited an initial increase in preference for protein, evident on day 45, that was followed by a significant rise in fat intake and fat preference (26% of total diet) by day 60 (Table 1). As in Experiment 1, body weight was strongly, positively correlated with protein intake on day 30 ($r=+0.71$, $p<0.01$) and day 45 ($r=+0.57$, $p<0.05$) but only with fat intake on day 60 ($r=+0.64$, $p<0.02$). The weight of the dissected fat pads, when expressed relative to body weight (% body fat), increased significantly only after puberty, from days 45 to 60 (Table 1), when it was positively correlated with preference for fat on day 45 ($r=+0.56$, $p<0.05$) and day 60 ($r=+0.74$, $p<0.01$) but not protein or carbohydrate. These age-related shifts in macronutrient intake, body weight and body fat accrual were accompanied by a rise in circulating hormones (Table 1). Levels of E_2 increased significantly from days 30 to 45 (+32%) but not thereafter, while P4 increased both from days 30 to 45 (+67%) and from days 45 to 60 (+140%). Smaller or more gradual changes were seen in the measure of insulin, which increased only on day 45, and of leptin, which reached significantly higher levels only on day 60 when % body fat also increased. The rise in both steroids from days 30 to 45 was accompanied by a marked increase in GAL peptide levels in the PVN and MPN, as well as the ME to which the GAL neurons project (Fig. 2), but little change in the ARC or other hypothalamic areas examined (Table 2). Significant, positive correlations (ranging from $r=+0.59$ to $r=+0.75$, $p<0.05$) on days 45 and 60 were consistently detected between PVN and MPN GAL and the measures of fat intake and P4, substantiating a close relationship between these parameters in pubertal females.

Experiment 3: Age-related changes in GAL and ENK mRNA in pubertal female rats

Building on the results showing marked changes in GAL peptide levels in the PVN and MPN around puberty, this experiment used real-time quantitative PCR to measure age-related changes in gene expression of GAL in these nuclei and also of the opioid peptide, ENK, which is similar to GAL in its relation to dietary fat and ovarian steroids (see Introduction). Similar to results in Experiments 1 and 2, the rats (n=30) tested on macronutrient diets and sacrificed at 30, 45 and 60 days of age (n=10/age) exhibited a significant increase in preference for fat from 16% to 29% ($p<0.01$), a relatively stable preference for protein (40-45%) after day 30, and a small decline in carbohydrate preference from 42% to 32%. As with GAL peptide, the levels of GAL mRNA increased markedly from days 30 to 45 in both the PVN and MPN (Fig. 3). A similar age-related increase, even larger in magnitude, was observed in the expression of ENK in these nuclei, while both GAL and ENK mRNA subsequently declined by day 60.

In contrast to the PVN and MPN, these peptides in the ARC remained stable or declined across ages (Fig. 3). These measurements of peptide mRNA substantiate the changes in GAL peptide observed in Experiment 2 and suggest that ENK may function similarly to GAL in pubertal animals.

Experiment 4: Steroids and peptides in relation to natural preferences for fat after puberty onset

Building on the results of Experiments 1-3, this experiment in a larger set of pubertal rats at a single age (n=24) investigated whether the close association between fat, steroids and peptides seen with a rise in fat preference across the 3 ages is similarly detected in rats with naturally different fat preferences at the same age. These rats examined on day 50 were subgrouped (n=8/group) as “high-fat eaters” that consumed >25% fat and “low-fat eaters” that consumed <15% fat. The high-fat eaters, while consuming 8.7 kcal more fat and slightly less protein and carbohydrate than the low-fat eaters, remained similar to this group in their total caloric intake and measures of leptin, body weight and body fat pad weights (Table 3). These groups differed, however, in their measures of E_2 and P4, which were significantly elevated in the high-fat eaters, and of GAL mRNA levels in the PVN and MPN measured by *in situ* hybridization (Figs. 4 and 5), which were also higher in the high-fat eaters and consistently, positively correlated (ranging from r=+0.61 to r=+0.72, p<0.01) with the rats' preference for fat. No group differences in GAL mRNA were evident in the ARC (Fig. 4) or any of the other hypothalamic areas examined in Experiment 2 (data not shown). These analyses at a single age around puberty, when rats exhibited differential increases in fat preference, reveal the same fat-steroid-GAL relationship that was detected across the 3 ages in Experiments 2 and 3.

Experiment 5: Fat intake, steroids and peptides in relation to day of puberty onset

This experiment focused on events that occur prior to puberty and might impact on the day of its onset, as well as on long-term feeding patterns and body fat accrual. Published studies demonstrate that consumption of a high-fat diet elevates female steroids, advances puberty onset, and increases body weight at maturity (see Introduction). In the present report, we tested whether similar phenomena can occur in a free-choice paradigm that allows animals to express their natural macronutrient preferences. Female rats (n=24) were examined on the 3 macronutrient diets, and their precise day of VO was recorded and related to their preference for fat before, during and after this day and also to their measures of steroids, GAL and body fat accrual after puberty, on day 70. The animals were subgrouped (n=8/group) based on their day of VO, with “early VO rats” reaching puberty on days 30-34 and “late VO rats” on days 37-40. While the early VO rats were slightly heavier than the late VO rats on days 40-50 and consumed less protein, these subgroups were similar in body weight before and after puberty and in their measures of total caloric intake and carbohydrate intake at all ages (Table 4). Only fat intake was significantly higher in the early VO rats, with their preference for this macronutrient almost double that of the late VO rats (37% vs 20%, p<0.01). With the data plotted in relation to day of VO, this greater fat intake was evident up to 4 days before, inversely correlated with day of VO across the entire group (r=-0.63, p<0.01), and it continued well after puberty onset (Fig. 6). A possible relationship of this stronger fat preference and underlying mechanisms at puberty to different phenomena occurring at maturity was suggested by the unusually strong, positive correlations (ranging from r=+0.76 to r=+0.84, p<0.001) detected between fat intake before or at VO and fat intake on day 70. Consistent with results in Experiment 4, this stronger fat preference of the early VO rats was accompanied by elevated levels of E_2 and P4 and higher GAL mRNA in the PVN and MPN (Fig. 6). While only 12g heavier in body weight, these early VO rats on day 70 had significantly heavier fat pad weights and higher leptin levels (Fig. 6), measures that across the entire group were negatively correlated with the day of VO (r=-0.67 and r=-0.62, respectively, p<0.01). Thus, early puberty onset as indicated by the day of VO (days 30-34) is temporally linked to an increase in fat

preference before puberty, which in turn is positively associated with higher steroids and GAL after puberty, in addition to greater fat intake and body fat accrual.

Discussion

These results show a close relationship between dietary fat, female steroids and orexigenic peptides in the PVN and MPN, as a function of age around puberty, preference for dietary fat, and day of puberty onset.

Age-related changes in feeding, nutrient preferences and body weight

The results, revealing a surge in protein intake from weaning (day 21) until day 35 followed by an increase in fat preference from 15% around puberty onset (day 35) to 30% by day 70, are consistent with the findings of prior studies in female rats (2,3). In addition, they demonstrate that the pups' body weight and daily weight gain are closely, positively related to their intake of protein before puberty, in contrast to their intake of fat after puberty as preference for this macronutrient increased to >25% of total diet. These findings demonstrate a clear shift with age in the body's patterns of ingesting and presumably metabolizing the different macronutrients. The critical transition period is detected at puberty onset, around day 35, when preference for fat starts to rise.

Age-related changes in circulating hormones

This age-related increase in preference for fat occurred simultaneously with a marked change in circulating levels of the ovarian steroids. Both E_2 and P4 increased significant from days 30 to 45, while a further increase in P4 occurred from days 45 to day 60, consistent with prior studies (49). The measures of insulin and leptin revealed a more gradual or delayed rise, as previously described (50-53). Insulin showed only a small increase from days 30 to 45, while the rise in leptin was statistically significant only on day 60, when it was positively related to body fat accrual as reported in post-pubertal rats (54). With these multiple measures, the present study allowed us to relate the steroids and peptide hormones to the macronutrients ingested by the pubertal rats. The main finding was a significant, positive correlation between levels of P4 and the rats' intake specifically of fat. This association is consistent with the evidence in adult female rats, showing fat-preferring animals to have elevated levels of P4 (36,42). It is further supported by the additional finding that administration of P4 preferentially stimulates the ingestion of a fat-rich diet (42). Together, these results suggest that the natural rise in P4 occurring at puberty is causally related to the simultaneous increase in fat intake.

Age-related changes in GAL in the brain

The most dramatic change in endogenous GAL, a 3-fold increase in peptide levels, was seen from just before puberty (day 30) to shortly after puberty (day 45). This effect was seen in the MPN and ME, consistent with earlier studies of the MPOA and ME (55,56). It was also detected in the PVN, but not in 6 other hypothalamic areas examined, the ARC, VMN, DMN, LH, SCN and SON, where GAL remained stable across ages. These results provide clear evidence for the anatomical specificity of this marked increase in GAL peptide around puberty. This site-specific change in peptide levels from days 30 to 45 was substantiated by the measurements of GAL mRNA, which showed peak expression in the PVN and MPN on day 45. This change in GAL mRNA and peptide occurred at the same age as the increase in ovarian steroids and the intake and preference for fat, as carbohydrate and protein intake remained stable or declined. This relationship around puberty is consistent with results observed in adult female rats, which reveal a close, positive association between PVN and MPN GAL and individual patterns of fat intake (42) and a marked rise in these parameters during proestrous (36,38). It also agrees with evidence obtained in adult GAL knockout mice, which demonstrates a marked reduction in fat consumption and a reversal of this effect with central GAL injection (57). These similar

findings in the MPN and PVN, possibly reflecting a close anatomical connection between these two nuclei (9), clearly contrast with those obtained in the ARC, where steroid receptors exist (58,59), but the steroids as well as dietary fat have little effect on or sometimes suppress GAL expression (25,42,60). Further support for the positive association between fat ingestion, steroids and GAL in the PVN and MPN has been obtained in studies of gender comparisons. This evidence demonstrates that adult female rats have higher PVN and MPN GAL than males and exhibit a stronger preference for fat that contributes to their greater adiposity (2,42). A recent study in ovariectomized adult rats (42) provides support for a causal relationship between the ovarian steroids, dietary fat, and GAL expression in the PVN and MPN.

Age-related changes in ENK in the brain

In addition to GAL, measurements of the opioid peptide, ENK, showed a similar age-related change in gene expression around puberty. The mRNA levels of ENK in the PVN and MPN increased dramatically from days 30 to 45, during the period of puberty onset, and then declined by day 60. This is in contrast to the ARC, where ENK mRNA like GAL remained stable or showed a decline across ages. These findings agree with an earlier study that observed an increase in ENK immunoreactive fibers in the MPOA of female rats around day 40 (32). Further, similar to GAL, ENK shows gender differences that are consistent with the positive relationship between steroids, peptide and fat intake, with adult females having higher ENK in the POA compared to males (31,32) while exhibiting a stronger preference for fat (42). These results support a close relationship between mechanisms involving the ovarian steroids and opioid peptides that control macronutrient preferences in pubertal animals. Similar results might be expected from analyses of another orexigenic peptide, orexin, in the perifornical lateral hypothalamus, which is similar to GAL and ENK in its relationship to the steroids and dietary fat (25,61-63). However, neuropeptide Y in the arcuate nucleus differs from GAL, ENK and orexin, in being suppressed by fat consumption (64) and having a negative as well as positive relationship with the steroids (36,65).

Changes in steroids and peptides in relation to fat preference in pubertal females

These close associations between the fat intake, ovarian steroids, and orexigenic peptides observed in relation to age and gender were confirmed in pubertal female rats examined as a function of their natural preference for fat. Rats that consumed more fat shortly after puberty (on day 50) had higher levels of E_2 and P4 compared to low-fat eaters and elevated GAL peptide specifically in the PVN and MPN. These differences were evident even when these subgroups were equal in caloric intake and body weight, thus dissociating the fat-steroid-GAL relationship from these parameters. This relationship around puberty was confirmed by the strong, positive correlations detected between the measures of fat intake, P4 levels, and GAL peptide. Studies in adult female rats have revealed similar patterns in fat-preferring rats (2,32,36,37,42,66) and specifically during the proestrous phase of the female cycle when fat is the preferred macronutrient (36). The involvement of ovarian steroids in this relationship between dietary fat and GAL is underscored by the finding that ovariectomy attenuates the stimulatory effect of fat consumption on this orexigenic peptide (42).

Changes in fat intake, steroids and peptides in relation to early puberty onset

Animal studies demonstrate that pre-pubertal nutrition, in addition to weight gain and body fat, affects reproductive maturation and promotes early puberty onset (4,5). Female rats forced to consume a diet rich in fat, which increases caloric intake as well as body weight, show puberty onset around days 32-33, as compared to days 37-39 for rats on a low-fat diet (67-69). The present study, using a different feeding paradigm that provides a free choice of pure macronutrient diets, allowed rats to exhibit their natural nutrient preferences around puberty while maintaining normal daily intake. With frequent monitoring of macronutrient

intake and day of VO, the results showed that fat intake prior to puberty onset, up to 4 days before, was inversely correlated with the day of VO, while positively related to fat intake days and weeks after puberty. These results demonstrate that natural patterns of fat intake are established early and suggest that greater fat consumption in a free-choice feeding paradigm provides a signal for triggering early puberty onset similar to that caused by a single, fat-rich diet (67-69). Whereas there is evidence that total caloric intake and body weight are also involved in initiating puberty (4,5), the signal related specifically to dietary fat in a free-choice situation appears to act independently of these parameters, which were not significantly altered in the early VO rats. With strong, positive correlations detected between the amount of fat consumed before puberty and intake at maturity, it is likely that the mechanisms underlying this behavioral pattern at these different stages of life may be similar, involving elevated steroids and peptide expression in the PVN and MPN as detected in the early VO rats on day 70. These endocrine and neurochemical mechanisms may also contribute to the increase in body fat accrual exhibited by the early VO rats at maturity, indicating that the increased preference for fat before puberty, in addition to being an early determinant of puberty onset, may also be a predisposing factor that stimulates weight gain long after puberty.

Possible causal relationships between fat intake, steroids and peptides at puberty

Through measurements of fat intake, steroids and peptides, this study revealed similar changes in all parameters as a function of age before and after puberty, individual preferences for fat around puberty, and precise day of VO. These results demonstrate close, positive associations between fat intake, ovarian steroids, and both GAL and ENK in the PVN and MPN, suggesting their involvement in balancing processes of energy balance and reproductive functions in pubertal females. Published literature (see Introduction) supports a causal relationship between these different parameters, with dietary fat increasing levels of the steroids and both fat and the steroids stimulating orexigenic peptides specifically in the PVN and MPN. With fat intake rising up to 4 days before VO in the early VO rats that overeat fat as adults, this evidence leads us to propose that this early burst in fat intake, possibly triggered by genetic or early environmental factors, is an initial step in a cascade of events with long-term consequences. The ingestion of fat may enhance the rise in levels of E_2, which is necessary for puberty onset and together with fat may stimulate GAL and ENK in the PVN and MPN. Functioning within a positive feedback loop, this series of events, starting earlier in fat-preferring rats, may continue to cycle into adulthood. In addition to affecting reproductive function, these peptides may cause a further increase in fat intake, which after puberty may involve a rise in P4 in addition to E_2 and contribute to greater body fat accrual at maturity. While optimal for functions of reproduction, these phenomena may ultimately put females at greater future risk, particularly on a fat-rich diet, for disorders such as obesity and diabetes (4,6).

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References

1. Ojeda, SR.; Urbanski, HF. Puberty in the Rat. In: Knobil, E., et al., editors. The Physiology of Reproduction. Raven Press; New York: 1988. p. 1699-1737.
2. Leibowitz SF, Lucas DJ, Leibowitz KL, Jhanwar YS. Developmental patterns of macronutrient intake in female and male rats from weaning to maturity. Physiol Behav 1991;50:1167–1174. [PubMed: 1798771]
3. Jean C, Fromentin G, Tome D, Larue-Achagiotis C. Wistar rats allowed to self-select macronutrients from weaning to maturity choose a high-protein, high-lipid diet. Physiol Behav 2002;76:65–73. [PubMed: 12175590]

4. Jasik CB, Lustig RH. Adolescent obesity and puberty: the “perfect storm”. Ann N Y Acad Sci 2008;1135:265–279. [PubMed: 18574233]
5. Lee JM, Appugliese D, Kaciroti N, Corwyn RF, Bradley RH, Lumeng JC. Weight status in young girls and the onset of puberty. Pediatrics 2007;119:e624–630. [PubMed: 17332182]
6. Golub MS, Collman GW, Foster PM, Kimmel CA, Rajpert-De Meyts E, Reiter EO, et al. Public health implications of altered puberty timing. Pediatrics 2008;121(Suppl 3):S218–230. [PubMed: 18245514]
7. Wade GN, Schneider JE, Li HY. Control of fertility by metabolic cues. Am J Physiol 1996;270:E1–19. [PubMed: 8772468]
8. Bloch GJ, Eckersell C, Mills R. Distribution of galanin-immunoreactive cells within sexually dimorphic components of the medial preoptic area of the male and female rat. Brain Res 1993;620:259–268. [PubMed: 7690302]
9. Merchenthaler I, Lopez FJ, Negro-Vilar A. Anatomy and physiology of central galanin-containing pathways. Prog Neurobiol 1993;40:711–769. [PubMed: 7683433]
10. Merchenthaler I. Enkephalin-immunoreactive neurons in the parvicellular subdivisions of the paraventricular nucleus project to the external zone of the median eminence. J Comp Neurol 1992;326:112–120. [PubMed: 1479064]
11. Bhanot R, Wilkinson M. Opiatergic control of LH secretion is eliminated by gonadectomy. Endocrinology 1983;112:399–401. [PubMed: 6291922]
12. Mechenthaler I. Galanin and the neuroendocrine axes. Cell Mol Life Sci 2008;65:1826–1835. [PubMed: 18500643]
13. Bloch GJ, Butler PC, Kohlert JG. Galanin microinjected into the medial preoptic nucleus facilitates female- and male-typical sexual behaviors in the female rat. Physiol Behav 1996;59:1147–1154. [PubMed: 8737905]
14. Sinchak K, Mills RH, Eckersell CB, Micevych PE. Medial preoptic area delta-opioid receptors inhibit lordosis. Behav Brain Res 2004;155:301–306. [PubMed: 15364490]
15. Acosta-Martinez M, Etgen AM. The role of delta-opioid receptors in estrogen facilitation of lordosis behavior. Behav Brain Res 2002;136:93–102. [PubMed: 12385794]
16. Lopez FJ, Merchenthaler I, Ching M, Wisniewski MG, Negro-Vilar A. Galanin: a hypothalamic-hypophysiotropic hormone modulating reproductive functions. Proc Natl Acad Sci USA 1991;88:4508–4512. [PubMed: 1709744]
17. Siervogel RM, Demerath EW, Schubert C, Remsberg KE, Chumlea WC, Sun S, et al. Puberty and body composition. Horm Res 2003;60:36–45. [PubMed: 12955016]
18. Yun R, Dourmashkin JT, Hill JO, Gayles EC, Fried SK, Leibowitz SF. PVN galanin increases fat storage and promotes obesity by causing muscle to utilize carbohydrate more than fat. Peptides 2005;26:2265–2273. [PubMed: 15893855]
19. Barton C, Lin L, York DA, Bray GA. Differential effects of enterostatin, galanin and opioids on high-fat diet consumption. Brain Res 1995;702:55–60. [PubMed: 8846096]
20. Smith BK, York DA, Bray GA. Effects of dietary preference and galanin administration in the paraventricular or amygdaloid nucleus on diet self-selection. Brain Res Bull 1996;39:149–154. [PubMed: 8866690]
21. Leibowitz SF, Kim T. Impact of a galanin antagonist on exogenous galanin and natural patterns of fat ingestion. Brain Res 1992;599:148–152. [PubMed: 1283559]
22. Odorizzi M, Fernette B, Angel E, Burlet C, Tankosic P, Burlet A. Galanin receptor antagonists decrease fat preference in Brattleboro rat. Neuropharmacology 2002;42:134–141. [PubMed: 11750923]
23. Zhang M, Gosnell BA, Kelley AE. Intake of high-fat food is selectively enhanced by mu opioid receptor stimulation within the nucleus accumbens. J Pharmacol Exp Ther 1998;285:908–914. [PubMed: 9580643]
24. Welch CC, Kim EM, Grace MK, Billington CJ, Levine AS. Palatability-induced hyperphagia increases hypothalamic Dynorphin peptide and mRNA levels. Brain Res 1996;721:126–131. [PubMed: 8793092]
25. Chang GQ, Karatayev O, Davydova Z, Leibowitz SF. Circulating triglycerides impact on orexigenic peptides and neuronal activity in hypothalamus. Endocrinology 2004;145:3904–3912. [PubMed: 15117877]

26. Chang GQ, Karatayev O, Ahsan R, Gaysinskaya V, Marwil Z, Leibowitz SF. Dietary fat stimulates endogenous enkephalin and dynorphin in the paraventricular nucleus: role of circulating triglycerides. Am J Physiol Endocrinol Metab 2007;292:E561–570. [PubMed: 17283367]
27. Bloch GJ, Kurth SM, Akesson TR, Micevych PE. Estrogen-concentrating cells within cell groups of the medial preoptic area: sex differences and co-localization with galanin- immunoreactive cells. Brain Res 1992;595:301–308. [PubMed: 1281739]
28. Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S. Expression and neuropeptidergic characterization of estrogen receptors (ERalpha and ERbeta) throughout the rat brain: anatomical evidence of distinct roles of each subtype. J Neurobiol 1998;36:357–378. [PubMed: 9733072]
29. Simerly RB, Young BJ, Carr AM. Co-expression of steroid hormone receptors in opioid peptide-containing neurons correlates with patterns of gene expression during the estrous cycle. Brain Res Mol Brain Res 1996;40:275–284. [PubMed: 8872312]
30. Warembourg M, Jolivet A. Immunocytochemical localization of progesterone receptors in galanin neurons in the guinea pig hypothalamus. J Neuroendocrinol 1993;5:487–491. [PubMed: 8680415]
31. Holland KL, Norby LA, Micevych PE. Peripubertal ontogeny and estrogen stimulation of cholecystokinin and preproenkephalin mRNA in the rat hypothalamus and limbic system. J Comp Neurol 1998;392:48–57. [PubMed: 9482232]
32. Watson RE Jr, Hoffmann GE, Wiegand SJ. Sexually dimorphic opioid distribution in the preoptic area: manipulation by gonadal steroids. Brain Res 1986;398:157–163. [PubMed: 3801887]
33. Brann DW, Chorich LP, Mahesh VB. Effect of progesterone on galanin mRNA levels in the hypothalamus and the pituitary: correlation with the gonadotropin surge. Neuroendocrinology 1993;58:531–538. [PubMed: 7509463]
34. Rossmanith WG, Marks DL, Clifton DK, Steiner RA. Induction of galanin mRNA in GnRH neurons by estradiol and its facilitation by progesterone. J Neuroendocrinol 1996;8:185–191. [PubMed: 8730652]
35. Taylor JA, Goubillon ML, Broad KD, Robinson JE. Steroid control of gonadotropin-releasing hormone secretion: associated changes in pro-opiomelanocortin and preproenkephalin messenger RNA expression in the ovine hypothalamus. Biol Reprod 2007;76:524–531. [PubMed: 17151352]
36. 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;139:1771–1780. [PubMed: 9528961]
37. Simerly RB, McCall LD, Watson SJ. Distribution of opioid peptides in the preoptic region: immunohistochemical evidence for a steroid-sensitive enkephalin sexual dimorphism. J Comp Neurol 1988;276:442–459. [PubMed: 2903870]
38. Marks DL, Smith MS, Vrontakis M, Clifton DK, Steiner RA. Regulation of galanin gene expression in gonadotropin-releasing hormone neurons during the estrous cycle of the rat. Endocrinology 1993;132:1836–1844. [PubMed: 7681766]
39. Kumar MS, Chen CL, Muther TF. Changes in the pituitary and hypothalamic content of methionine-enkephalin during the estrous cycle of rats. Life Sci 1979;25:1687–1696. [PubMed: 574607]
40. Faure-Virelizier C, Croix D, Bouret S, Prevot V, Reig S, Beauvillain JC, et al. Effects of estrous cyclicity on the expression of the galanin receptor Gal-R1 in the rat preoptic area: a comparison with the male. Endocrinology 1998;139:4127–4139. [PubMed: 9751492]
41. Mitchell V, Lecompte F, Beauvillain JC. Regulation of galanin receptor GalR1 mRNA expression by ovarian steroids in oestrogen receptor alpha-immunoreactive neurones: identification of distinct populations of neurones in the preoptic area. J Neuroendocrinol 2004;16:138–145. [PubMed: 14764000]
42. Leibowitz SF, Akabayashi A, Wang J, Alexander JT, Dourmashkin JT, Chang GQ. Increased caloric intake on a fat-rich diet: role of ovarian steroids and galanin in the medial preoptic and paraventricular nuclei and anterior pituitary of female rats. J Neuroendocrinol 2007;19:753–766. [PubMed: 17850457]
43. Gray JM, Greenwood MR. Time course of effects of ovarian hormones on food intake and metabolism. Am J Physiol 1982;243:E407–412. [PubMed: 7137344]
44. Wu AH, Pike MC, Stram DO. Meta-analysis: dietary fat intake, serum estrogen levels, and the risk of breast cancer. J Natl Cancer Inst 1999;91:529–534. [PubMed: 10088623]

45. Dourmashkin JT, Chang GQ, Gayles EC, Hill JO, F SK, Julien C, Leibowitz SF. Different forms of obesity as a function of diet composition. Int J Obes 2005;29:1368–1378.
46. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Academic Press; Sydney: 1986.
47. Akabayashi A, Zaia CT, Koenig JI, Gabriel SM, Silva I, Leibowitz SF. Diurnal rhythm of galanin-like immunoreactivity in the paraventricular and suprachiasmatic nuclei and other hypothalamic areas. Peptides 1994;15:1437–1444. [PubMed: 7535427]
48. Chang GQ, Gaysinskaya V, Karatayev O, Leibowitz SF. Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. J Neurosci 2008;28:12107–12119. [PubMed: 19005075]
49. Ojeda, SR.; U, H. F Puberty in rat. In: Knobil, E.; N, JD., editors. Physiology of Reproduction. Vol. 2nd. Raven Press; New York: 1994. p. 363-397.
50. Potau N, Ibanez L, Rique S, Carrascosa A. Pubertal changes in insulin secretion and peripheral insulin sensitivity. Hormone Research 1997;48:219–226. [PubMed: 9362392]
51. Bloch CA, Clemons P, Sperling MA. Puberty decreases insulin sensitivity. The Journal of pediatrics 1987;110:481–487. [PubMed: 2950219]
52. Cheung CC, Hohmann JG, Clifton DK, Steiner RA. Distribution of galanin messenger RNA-expressing cells in murine brain and their regulation by leptin in regions of the hypothalamus. Neuroscience 2001;103:423–432. [PubMed: 11246157]
53. Smith JT, Waddell BJ. Developmental changes in plasma leptin and hypothalamic leptin receptor expression in the rat: peripubertal changes and the emergence of sex differences. J Endocrinol 2003;176:313–319. [PubMed: 12630916]
54. Zeinoaldini S, Swarts JJ, Van de Heijning BJ. A signaling role for leptin in puberty onset in female rats? J Pediatr Endocrinol Metab 2006;19:1239–1247. [PubMed: 17172085]
55. Marks DL, Lent KL, Rossmanith WG, Clifton DK, Steiner RA. Activation-dependent regulation of galanin gene expression in gonadotropin-releasing hormone neurons in the female rat. Endocrinology 1994;134:1991–1998. [PubMed: 7512492]
56. Gabriel SM, Kaplan LM, Martin JB, Koenig JI. Tissue-specific sex differences in galanin-like immunoreactivity and galanin mRNA during development in the rat. Peptides 1989;10:369–374. [PubMed: 2474154]
57. Adams AC, Clapham JC, Wynick D, Speakman JR. Feeding behaviour in galanin knockout mice supports a role of galanin in fat intake and preference. J Neuroendocrinol 2008;20:199–206. [PubMed: 18088361]
58. Mills RH, Romeo HE, Lu JK, Micevych PE. Site-specific decrease of progesterone receptor mRNA expression in the hypothalamus of middle-aged persistently estrus rats. Brain Res 2002;955:200–206. [PubMed: 12419537]
59. Henry WW Jr, Medlock KL, Sheehan DM, Scallet AC. Detection of estrogen receptor (ER) in the rat brain using rat anti-ER monoclonal IgG with the unlabeled antibody method. Histochemistry 1991;96:157–162. [PubMed: 1917572]
60. Leibowitz SF, Wortley KE. Hypothalamic control of energy balance: different peptides, different functions. Peptides 2004;25:473–504. [PubMed: 15134868]
61. Wortley KE, Chang GQ, Davydova Z, Leibowitz SF. Peptides that regulate food intake: orexin gene expression is increased during states of hypertriglyceridemia. Am J Physiol Regul Integr Comp Physiol 2003;284:R1454–R1465. [PubMed: 12560202]
62. Small CJ, Goubillon ML, Murray JF, Siddiqui A, Grimshaw SE, Young H, et al. Central orexin A has site-specific effects on luteinizing hormone release in female rats. Endocrinology 2003;144:3225–3236. [PubMed: 12810579]
63. Porkka-Heiskanen T, Kalinchuk A, Alanko L, Huhtaniemi I, Stenberg D. Orexin A and B levels in the hypothalamus of female rats: the effects of the estrous cycle and age. European journal of endocrinology / European Federation of Endocrine Societies 2004;150:737–742. [PubMed: 15132733]
64. Leibowitz SF, Akabayashi A, Wang J. Obesity on a high-fat diet: role of hypothalamic galanin in neurons of the anterior paraventricular nucleus projecting to the median eminence. J Neurosci 1998;18:2709–2719. [PubMed: 9502828]

65. Hill JW, Elmquist JK, Elias CF. Hypothalamic pathways linking energy balance and reproduction. Am J Physiol Endocrinol Metab 2008;294:E827–832. [PubMed: 18285524]

66. Bloch GJ, Butler PC, Eckersell CB, Mills RH. Gonadal steroid-dependent GAL-IR cells within the medial preoptic nucleus (MPN) and the stimulatory effects of GAL within the MPN on sexual behaviors. Ann N Y Acad Sci 1998;863:188–205. [PubMed: 9928171]

67. Boukouvalas G, Antoniou K, Papalexi E, Kitraki E. Post weaning high fat feeding affects rats' behavior and hypothalamic pituitary adrenal axis at the onset of puberty in a sexually dimorphic manner. Neuroscience 2008;153:373–382. [PubMed: 18378403]

68. Kirtley D, Maher R. Effect of an isocaloric high fat diet on initiation of puberty in Osborne-Mendel rats. Biol Reprod 1979;21:331–338. [PubMed: 573636]

69. Frisch RE, Hegsted DM, Yoshinaga K. Body weight and food intake at early estrus of rats on a high-fat diet. ProcNatlAcadSciUSA 1975;72:4172–4176.

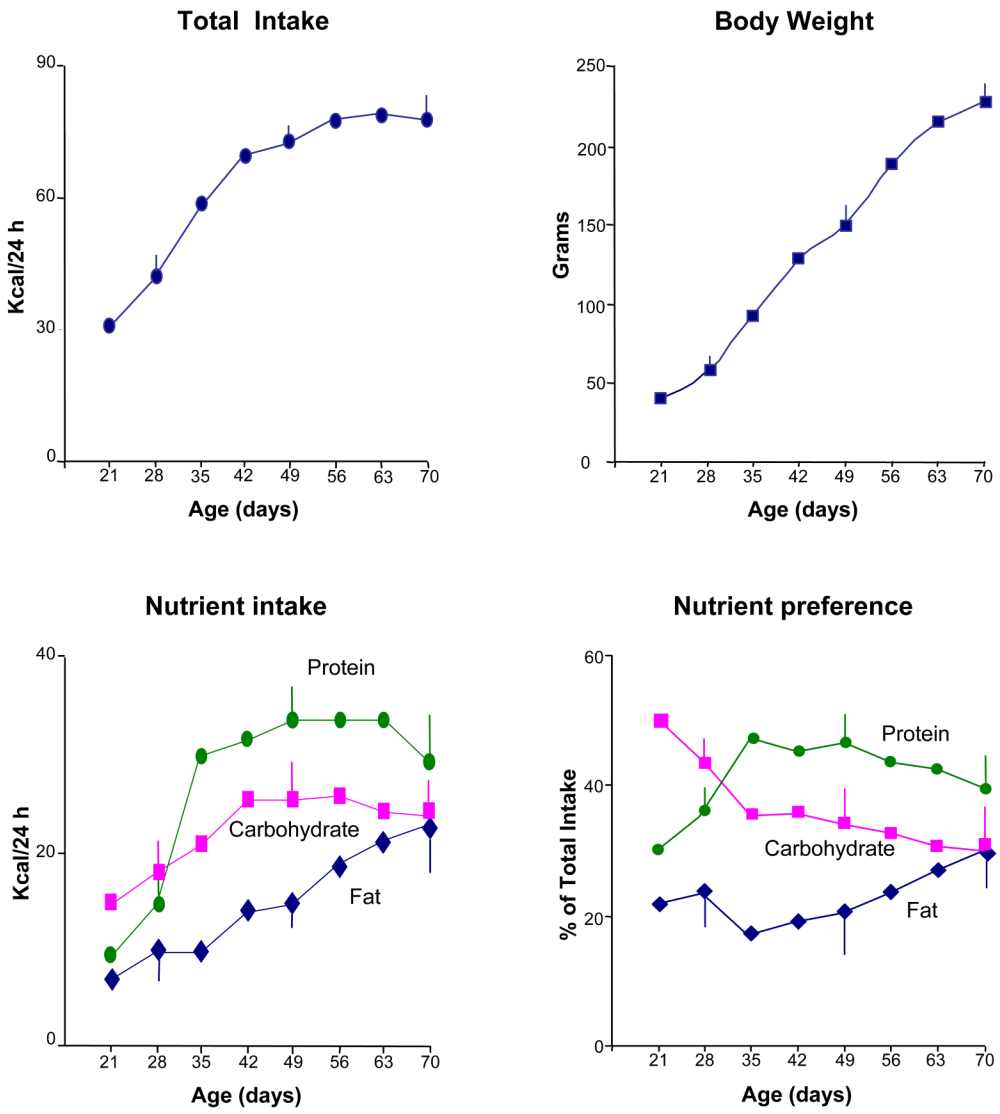

Figure 1.

Measures of total intake, body weight and macronutrient intake and preference (% of total diet) across ages, from weaning (day 21) to maturity (day 70), in female rats maintained on 3 diets consisting of pure fat, protein and carbohydrate (Experiment 1). Given are means ± SEM.

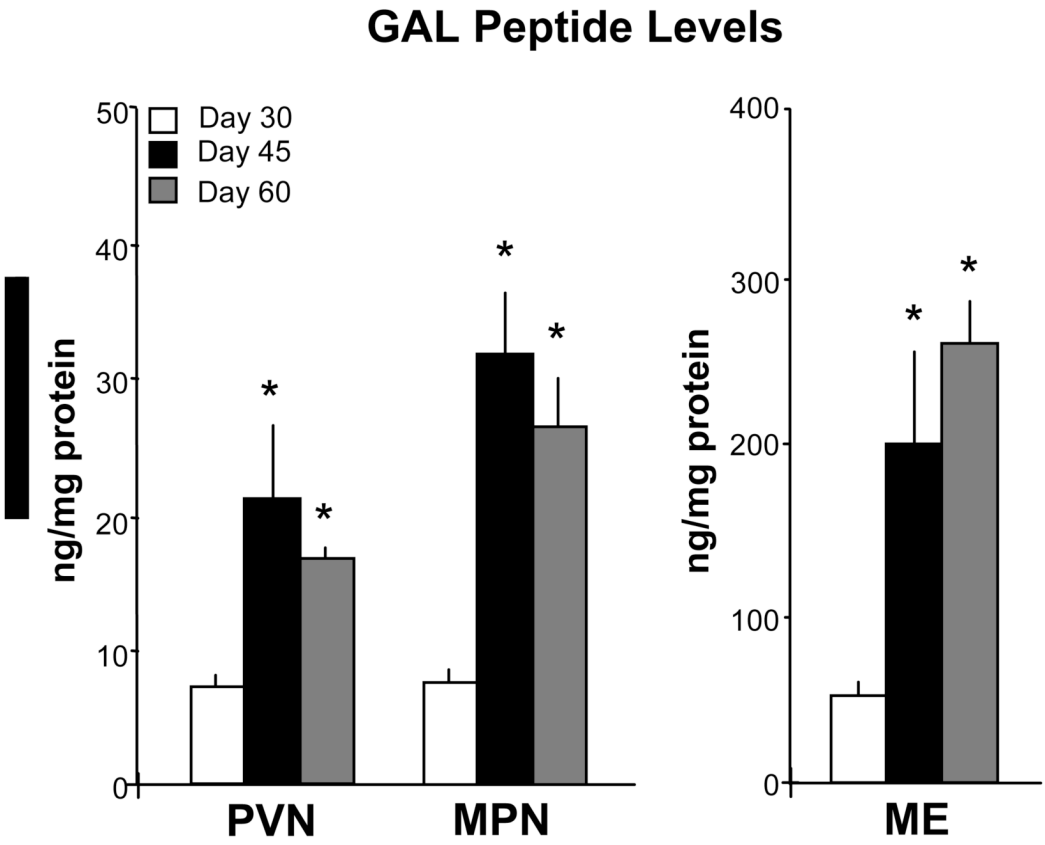

Figure 2.
Galanin (GAL) peptide levels in different areas showing significant differences as a function of age, shortly before puberty (day 30), after puberty (day 45), and at maturity (day 60) (Experiment 2). Given are means ± SEM. On day 45, female rats showed a dramatic increase in GAL peptide levels in the paraventricular nucleus (PVN), medial preoptic nucleus (MPN), and median eminence (ME), but not in other hypothalamic areas shown in Table 2. *, $p<0.05$ for direct comparisons between GAL levels on days 45 or 60 and on day 30.

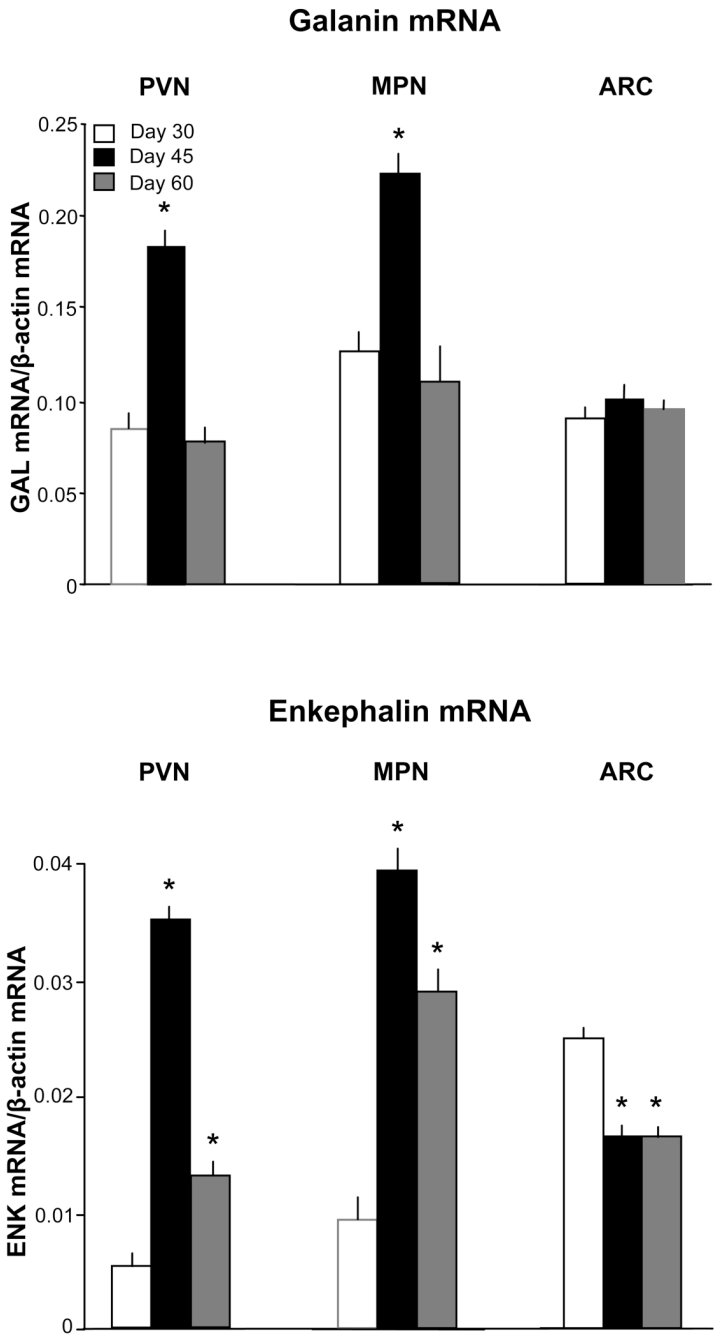

Figure 3.

Galanin (GAL) and enkephalin (ENK) mRNA levels (measured by real-time quantitative PCR) in the paraventricular nucleus (PVN) and medial preoptic nucleus (MPN) showing significant differences as a function of age, measured shortly before puberty (day 30), after puberty (day 45), and at maturity (day 60) (Experiment 3). Given are means ± SEM. Female rats on day 45 showed a marked increase in GAL and ENK mRNA in the PVN and MPN but not arcuate nucleus (ARC). *, $p<0.05$ for direct comparisons between GAL or ENK mRNA on days 45 or 60 and on day 30.

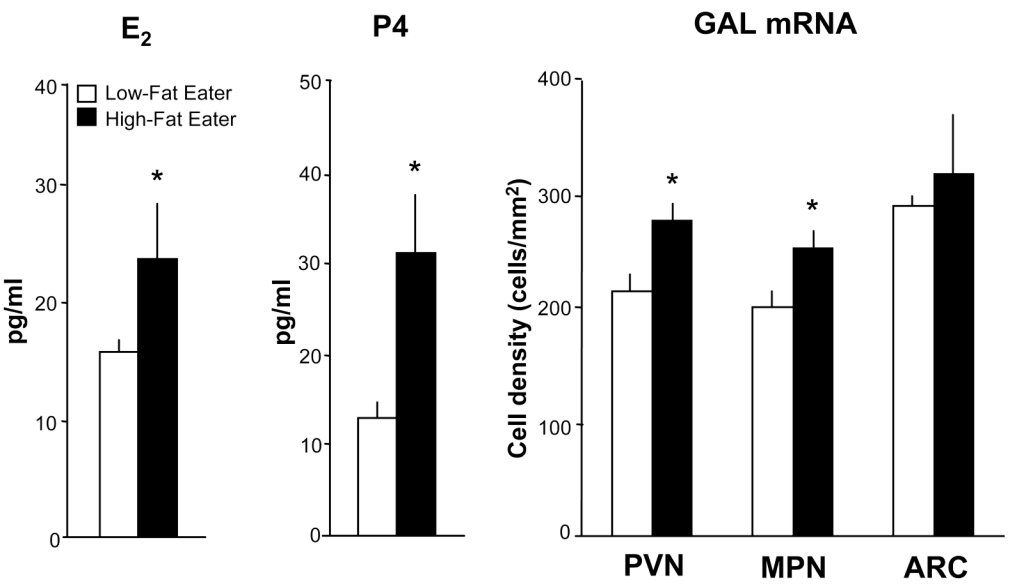

Figure 4.

Measurements of circulating levels of 17β-oestradiol (E_2), progesterone (P4) and galanin (GAL) mRNA (measured via *in situ* hybridization) in different brain areas of post-pubertal rats (day 50) subgrouped as high-fat or low-fat eaters (Experiment 4). Given are means ± SEM. Female rats that exhibit a preference for fat at puberty had significantly higher E_2 and P4 levels and also GAL mRNA in the paraventricular nucleus (PVN) and medial preoptic nucleus (MPN) but not arcuate nucleus (ARC). *, $p<0.05$ for direct comparisons between measures in high-fat compared to low-fat eaters.

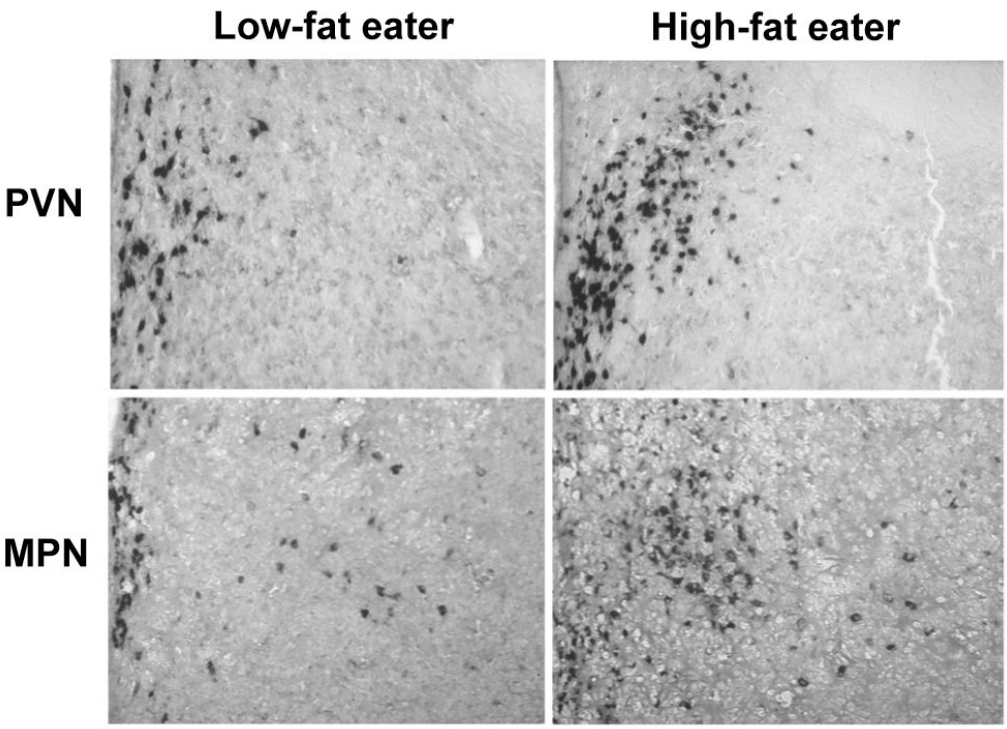

Figure 5.
Photomicrographs of GAL mRNA in the paraventricular nucleus (PVN) or medial preoptic nucleus (MPN) (magnification 100×) of low-fat and high-fat eaters. (See data presented in Figure 4).

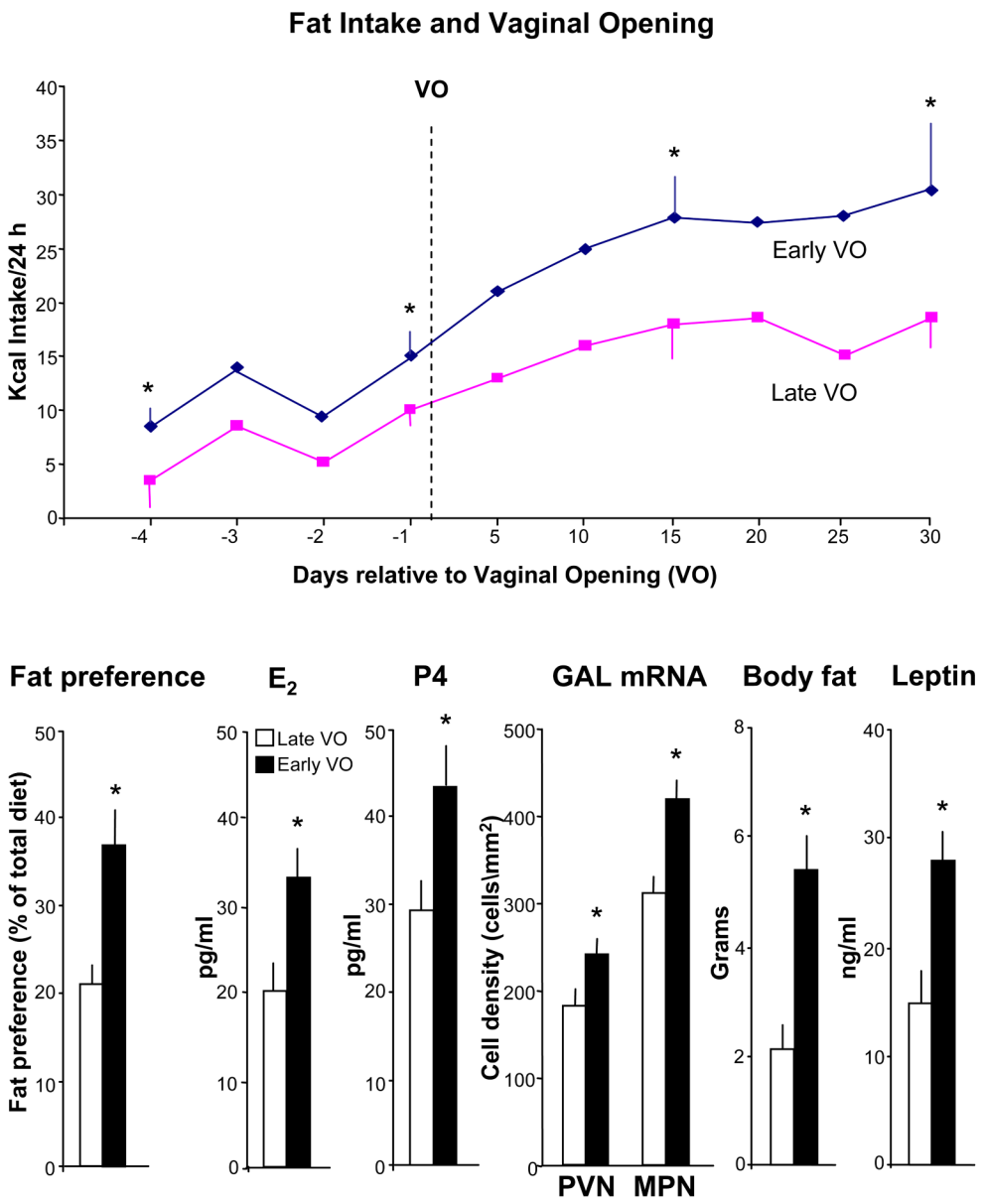

Figure 6.

Daily fat intake (kcal) from 4 days before and 30 days after vaginal opening (V0), with measurements of fat preference, 17β-oestradiol (E_2) and progesterone (P4), galanin (GAL) mRNA in the paraventricular nucleus (PVN) and medial preoptic nucleus (MPN), body fat and leptin levels on day 70. Given are means ± SEM. The early VO rats had significantly greater fat intake from 4 days before VO. This measure of intake before puberty was positively correlated with fat intake at maturity (day 70), when the early VO rats exhibited elevated levels of E_2 and P4, higher GAL mRNA in the PVN and MPN, and greater body fat and leptin levels. *, $p<0.05$ for direct comparisons between early and late VO rats.

Table 1

Nutrient intake, body weight and hormones in female rats at 30, 45, and 60 days of age (Experiment 2).

	Day 30	Day 45	Day 60
Nutrient intake (kcal)			
Protein	12.9 ± 2.4	31.6 ± 2.7*	35.3 ± 4.0**
Fat	7.2 ± 2.2	14.2 ± 1.3*	21.6 ± 2.9***
Carbohydrate	16.2 ± 3.5	24.5 ± 3.1*	26.3 ± 5.2**
Total	36.3 ± 3.3	70.3 ± 1.9*	83.2 ± 3.7***
Nutrient preference (% of total intake)			
Protein	35.5 ± 5.1	45.0 ± 3.6*	42.4 ± 4.7
Fat	19.8 ± 3.0	20.2 ± 2.4	26.0 ± 2.6***
Carbohydrate	44.6 ± 6.7	34.9 ± 4.9	31.6 ± 7.8
Body weight (g)	57 ± 5.2	143 ± 5.5*	209 ± 8.3*
Body weight gain (g/day)	4.0 ± 0.4	5.8 ± 0.7*	4.1 ± 1.0*
Total body fat (g)	1.8 ± 0.1	4.2 ± 0.4*	8.2 ± 0.7*
% body fat (g/g body weight)	3.2 ± 0.1	3.0 ± 0.2	3.9 ± 0.2***
17β-Oestradiol (pg/ml)	15.2 ± 1.1	20.0 ± 2.5*	21.8 ± 3.9**
Progesterone (pg/ml)	10.1 ± 2.9	16.9 ± 3.1*	40.5 ± 7.3***
Insulin (ng/ml)	0.97 ± 0.1	1.36 ± 0.3*	1.53 ± 0.3**
Leptin (ng/ml)	1.96 ± 0.3	2.44 ± 0.3	3.20 ± 0.6**

* $p < 0.05$ for direct comparisons between days 45 and 30 or days 60 and 45

** $p < 0.05$ for direct comparisons between days 60 and 30

*** $p < 0.05$ for direct comparisons between day 60 and days 45 and 30

Table 2

Galanin peptide levels (ng/mg protein) in hypothalamic areas showing no changes as a function of age (Experiment 2).

	Day 30	Day 45	Day 60
ARC	15.2 ± 3.0	20.3 ± 5.7	17.8 ± 3.5
VMH	7.2 ± 2.3	8.1 ± 2.2	9.4 ± 2.8
DMN	66.5 ± 9.5	70.5 ± 8.1	65.0 ± 8.1
SCN	38.8 ± 6.0	50.4 ± 5.6	49.9 ± 8.9
SON	11.5 ± 2.3	13.2 ± 2.0	15.6 ± 1.9
LH	3.5 ± 0.6	4.9 ± 1.6	4.7 ± 0.8

Abbreviations: ARC, arcuate nucleus; VMH, ventromedial hypothalamus; DMN, dorsomedial nucleus; SCN, suprachiasmatic nucleus; SON, suprachiasmatic nucleus; SON, supraoptic nucleus; LH, lateral hypothalamus.

Table 3

Measures in pubertal female rats subgrouped (Day 50) as High-fat eaters (>25%) and Low-fat eaters (<15%) (Experiment 4).

	Low-fat eaters	High fat-eaters
Fat Intake (kcal)	12.3 ± 2.4	21.0 ± 3.5*
Protein Intake (kcal)	36.2 ± 1.5	33.6 ± 3.8
Carbohydrate Intake (kcal)	27.0 ± 5.5	23.2 ± 2.8
Total Intake (kcal)	75.5 ± 1.5	77.8 ± 2.8
Leptin (ng/ml)	1.74 ± 0.4	2.63 ± 0.6
Body Weight (g)	147 ± 6.7	156 ± 3.8
Body Fat (g)	5.0 ± 1.5	7.6 ± 1.8

* $p < 0.05$ for direct comparisons between high-fat and low-fat eaters.

Table 4

Body weight and nutrient intake at different ages in female rats subgrouped as late VO (Days 37-40) or early VO (Days 30-34) rats (Experiment 5).

	Late VO	Early VO
Body Weight (g)		
Days 25-30	50 ± 3.5	55 ± 3.6
Days 40-50	134 ± 4.7	147 ± 5.1*
Days 60-70	219 ± 6.3	231 ± 7.2
Total Intake (kcal)		
Days 25-30	34 ± 6.4	40 ± 6.1
Days 40-50	74 ± 7.5	74 ± 9.7
Days 60-70	84 ± 6.9	81 ± 7.6
Fat Intake (kcal)		
Days 25-30	6 ± 1.5	11 ± 1.8*
Days 40-50	17 ± 1.7	28 ± 3.2*
Days 60-70	18 ± 3.7	30 ± 3.8*
Protein Intake (kcal)		
Days 25-30	12 ± 2.6	15 ± 2.8
Days 40-50	35 ± 2.3	27 ± 3.7*
Days 60-70	47 ± 4.2	35 ± 4.6*
Carbohydrate Intake (kcal)		
Days 25-30	16 ± 2.3	14 ± 1.7
Days 40-50	22 ± 3.5	19 ± 3.2
Days 60-70	19 ± 2.4	16 ± 3.1

* $p < 0.05$ for direct comparisons between early VO and late VO rats.