Published in final edited form as:

Neuropeptides. 2016 August ; 58: 103–109. doi:10.1016/j.npep.2016.01.004.

The effects of high fat diet and estradiol on hypothalamic prepro-QRFP mRNA expression in female rats

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Abstract

Estradiol (E2) is a potent regulator of feeding behavior, body weight and adiposity in females. The hypothalamic neuropeptide, QRFP, is an orexigenic peptide that increases the consumption of high fat diet (HFD) in intact female rats. Therefore, the goal of the current series of studies was to elucidate the effects of E2 on the expression of hypothalamic QRFP and its receptors, QRFP-r1 and QRFP-r2, in female rats fed a HFD. Alterations in prepro-QRFP, QRFP-r1, and QRFP-r2 expression across the estrous cycle, following ovariectomy (OVX) and following estradiol benzoate (EB) treatment were assessed in the ventral medial nucleus of the hypothalamus/arcuate nucleus (VMH/ARC) and the lateral hypothalamus. In intact females, consumption of HFD increased prepro-QRFP and QRFP-r1 mRNA levels in the VMH/ARC during diestrus, a phase associated with increased food intake and low levels of E2. To assess the effects of diminished endogenous E2, rats were ovariectomized. HFD consumption and OVX increased prepro-QRFP mRNA in the VMH/ARC. Ovariectomized rats consuming HFD expressed the highest levels of QRFP. In the third experiment, all rats received EB replacement every 4 days following OVX to examine the effects of E2 on QRFP expression. Prepro-QRFP, QRFP-r1 and QRFP-r2 mRNA were assessed prior to and following EB administration. EB replacement significantly reduced prepro-QRFP mRNA expression in the VMH/ARC. Overall these studies support a role for E2 in the regulation of prepro-QRFP mRNA in the VMH/ARC and suggest that E2's effects on food intake may be via a direct effect on the orexigenic peptide, QRFP.

Keywords

QRFP; Estradiol; High fat diet; mediobasal hypothalamus

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Introduction

Dietary fat is critical for overall health, development and reproductive function. However, overweight/obese individuals may experience numerous physiological and psychological comorbidities (Friedman and Kim, 1985; Linne, 2004; Nelson and Fleming, 2007; Norman and Clark, 1998; Wade and Jones, 2004). Though many of these comorbidities are prevalent in both males and females, obesity in females is associated with a higher rate of infertility, Type II Diabetes, cardiovascular disease, depression, and some forms of cancer (Guh et al., 2009; Hackethal et al., 2014; Yao et al., 2014). Unlike their male counterparts, obesity rates in females continue to rise throughout the lifespan, with approximately 42% of post-menopausal women being considered obese (NHANES). Due to a dramatic increase in life expectancy, women will spend the second half of their lives in estrogen deficiency, which predisposes them to the development of visceral obesity, metabolic syndrome, insulin resistance and Type II Diabetes (Carr, 2003; Ley et al., 1992; Louet et al., 2004; Mauvais-Jarvis, 2012; Mauvais-Jarvis et al., 2013; Panotopoulos et al., 1996; Phillips et al., 2008; Rosety-Rodriguez et al., 2013).

Numerous studies have investigated the role of endogenous estrogens on energy homeostasis. Data from rodent models indicate that females consume more calories during diestrus than proestrus or estrus, with rats in estrus consuming the fewest calories (Asarian and Geary, 2006, 2013; Wade, 1972). Marked decreases in endogenous estrogen and progesterone via ovariectomy (OVX) induce hyperphagia and rapid increases in weight and adiposity, and increases in meal size, serum glucose, cholesterol, triglycerides and free fatty acids (Eckel, 1999; Eckel and Geary, 1999; Eckel et al., 2000; Eckel and Moore, 2004; Majumdar et al., 2014; McElroy and Wade, 1987; Messina et al., 2006; Santollo and Eckel, 2008a; Schneider et al., 1986; Wade, 1975; Wade and Schneider, 1992). Studies investigating the effects of estrous cycle, OVX and estradiol benzoate (EB) treatment on the feeding effects of various peptides and drugs have illustrated the complex role that estradiol (E2) plays on energy homeostasis (Brown and Clegg, 2010; Eckel, 1999; Eckel et al., 2002; Eckel et al., 2005; Messina et al., 2006; Rivera and Eckel, 2005; Rivera et al., 2009; Santollo and Eckel, 2008a, b). Additionally, studies have reported effects of estrous cycle and E2 treatment on the expression of hypothalamic peptides involved in feeding behavior (Gao et al., 1997; Khorram et al., 1985; Le et al., 1991; Leibowitz et al., 1998; Olofsson et al., 2009; Petersen et al., 1993; Silva et al., 2010; Vigo et al., 2007a; Vigo et al., 2007b; Zhao et al., 2005).

QRFP (pyroglutamylated arginine-phenylalanine amide) is a neuropeptide expressed primarily in the ventromedial hypothalamus (VMH), arcuate nucleus (ARC) and the lateral hypothalamus (LH) (Chartrel et al., 2003; Fukusumi et al., 2006; Kampe et al., 2006), which are important brain regions for the regulation of ingestive behaviors and are abundant in neurotransmitters, neuropeptides, and receptor systems that influence feeding (Arora and Anubhuti, 2006; Kageyama et al., 2012; Qi et al., 2015). Studies focusing on the effects of QRFP on feeding behavior have demonstrated an orexigenic effect of QRFP, thereby supporting a role for QRFP in the regulation of dietary fat and the development of obesity (Chartrel et al., 2003; do Rego et al., 2006; Moriya et al., 2006; Primeaux, 2011; Primeaux et al., 2013; Primeaux et al., 2008; Takayasu et al., 2006; Tobari et al., 2011; Ukena et al.,

2010; Zagorácz et al., 2015). The QRFP precursor generates a 26-amino acid peptide (QRFP-26, also referred to as 26RFa) and an N-terminal extended form of 43-amino acids (QRFP-43, also referred to as 43RFa). Central administration of QRFP-26 selectively increases high fat diet (HFD) intake, without altering low fat diet (LFD) intake in intact female rats (Primeaux, 2011; Primeaux et al., 2013). Both QRFP-26 and QRFP-43 are potent ligands for the G protein-coupled receptors, QRFP-r1 and QRFP-r2 (GPR103a and GPR103b in mouse), which are expressed throughout the brain (Chartrel et al., 2003; do Rego et al., 2006; Findeisen et al., 2011; Lectez et al., 2009; Moriya et al., 2006; Navarro et al., 2006; Patel et al., 2008; Primeaux, 2011; Primeaux et al., 2013; Primeaux et al., 2008; Takayasu et al., 2006; Ukena et al., 2014; Ukena et al., 2010; Ukena et al., 2013; Ukena et al., 2011). Within appetite regulation centers of the hypothalamus, QRFP-r1 is primarily expressed in the VMH/ARC, while QRFP-r2 is primarily expressed in the LH (Leprince et al., 2013). Prepro-QRFP mRNA expression in the VMH/ARC fluctuates across the estrous phase in chow-fed female rats (Primeaux, 2011).

The goal of the current series of experiments was to examine the effects of E2 on hypothalamic prepro-QRFP, QRFP-r1 (VMH/ARC) and QRFP-r2 (LH) mRNA expression in female rats fed either a HFD or a LFD. Alterations in expression across the estrous cycle, following OVX and following EB replacement were assessed in the VMH/ARC and the LH.

Materials and Methods

Animals

Adult female Long Evans rats (Harlan, Inc., Indianapolis, IN) weighing between 150–175g upon arrival were used in these experiments. Rats were housed in standard shoebox cages in an AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care) approved animal facility on a 12/12h light/dark cycle (lights on at 0700) with food and water available *ad libitum*. Rats were given continuous access to a pelleted high fat diet (HFD, 60% kcal from fat; D12492; Research Diets, New Brunswick, NJ) or a pelleted low fat diet (LFD, 10% kcal from fat; D12450B; Research Diets). All procedures were approved by the Pennington Biomedical Research Center and LSU Health Sciences Center Institutional Animal Care and Use Committees.

Experiment 1: Influence of estrous cycle on hypothalamic gene expression

Determinations of estrous cycle began 2 weeks following access to either the HFD or LFD diet. During this time, body weight and food intake was measured. Daily vaginal smears were taken via lavage from cycling female rats between 0900 and 1000 for 2 weeks. Estrous phase determination was based on vaginal smear cytology of cells viewed under a low-power microscope as previously described (Primeaux, 2011). Briefly, diestrus was identified by the presence of leukocytes and nucleated epithelial cells. Proestrus was identified by large clusters of round nucleated cells and the absence of leukocytes. Estrus was identified by the presence of cornified cells. Females were euthanized following determination of estrous cycle and estrous phase was verified by visual inspection of the reproductive organs (n=7–13/phase/diet). Brains were removed, frozen on dry ice and stored at –80°C until further processing.

Experiment 2: Effect of OVX on hypothalamic gene expression

For OVX, female rats were anesthetized with isoflurane (1.5–3% in oxygen) immediately prior to surgery. The dorsal flank regions of the rat were shaved, cleaned and injected with a local anesthetic (Lidacaine/Bupivacaine; 1mg/kg, sc) to reduce discomfort. Ovaries were removed through small bilateral dorsal flank incisions. Once the ovaries were removed, muscle incisions were sutured with sterile absorbable (Vicryl) sutures and skin incisions were closed with wound clips. Following surgery, Carprofen (1.0 mg/kg, sc) was given for postoperative analgesia. Rats were fed either the HFD or LFD beginning 2 weeks following OVX surgery. Pair-fed groups were included to control for hyperphagia associated with OVX (OVX-PF). Daily caloric intake for the OVX-PF groups was based on the daily HFD and LFD intake from cycling rats in Experiment 1. Body weight and food intake were measured for 3 weeks. Removal of the ovaries was verified at the time of sacrifice (n=8–9/group/diet) and brains were removed, frozen on dry ice and stored at –80°C until further processing.

Experiment 3: Hypothalamic gene expression following EB treatment

Female rats underwent OVX surgery as described in Experiment 2. Rats were habituated to the HFD or LFD beginning 1 week prior to OVX surgery. Beginning 1 week following surgery, estradiol benzoate (EB, 4µg/100µl, sc, @ 1500h) was administered to all rats every 4 days to mimic the rat estrous cycle. Food intake and body weight were assessed daily for 3 weeks. Rats were sacrificed either at 0900h on the day in which they were scheduled to receive EB (pre-EB group) or at 0900h on the day following EB (EB group) administration. The removal of the ovaries was verified at the time of sacrifice (n=8–10/group/diet) and brains were collected, frozen and stored at –80°C until further processing.

Real-time Polymerase Chain Reaction (PCR)

All brains were processed for Real Time PCR. For gene expression analysis, frozen brains were sliced using a freezing microtome (Microm HM400, Waldorf, Germany) and beginning at AP –2.3 (based on the Rat Brain Atlas by Paxinos and Watson (Paxinos and Watson, 1997), bilateral 1mm diameter brain punches, approximately 2mm thick, were taken from the VMH/ARC and LH. Punches were immediately placed in a 1.5ml microfuge tube on dry ice and stored at –80°C until further processing. RNA was isolated using Tri-Reagent (Molecular Research Ctr, Cincinnati, OH USA) and RNeasy Minikit procedures (Qiagen, Valencia, CA USA) and based on previous experiments by Primeaux et al. (Primeaux, 2011; Primeaux et al., 2007; Primeaux et al., 2008; Primeaux et al., 2006). Reverse transcription (RT) was conducted using the High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems/Life Technologies, Foster City, CA, USA). For RT, 1.0µg of RNA from each sample was added to random primers (10×), dNTP (25×), MultiScribe Reverse Transcriptase (50U/µl) and RT buffer (10×) and incubated in a thermal cycler (PTC-100, MJ Research, Inc, Watertown, MA, USA) for 10 min at 27°C, then for 120 min at 37°C. Taqman Gene Expression Assays (Applied Biosystems/Life Technologies) were used to assess levels of prepro-QRFP mRNA (Rn01644297_s1), QRFP-r1 (Rn01644293_mH), QRFP-r2 (Rn01425102_m1) and the housekeeping gene, cyclophilin (Rn00452692_m1). For Real-time PCR, Taqman Universal PCR Master Mix (Applied Biosystems), gene expression

assay, and RT product (10ng) were added to a 384 well plate. The cycling parameters consisted of an initial 2 min incubation at 50 °C, followed by 10 min at 95 °C, then 15 sec at 95 °C, and a 1 min annealing/extension step at 60 ° (40 cycles). The quantity of prepro-QRFP mRNA expression was based on a standard curve and normalized to cyclophilin levels (ABI Prism 7900 Sequence Detection System, Applied Biosystems). Data is expressed as fold-change from the LFD fed control for each experiment.

Statistical Analysis

In Experiment 1, hypothalamic gene expression was assessed by a 2×3 ANOVA (diet $\times$ estrous phase). Average daily food intake in kilocalories (kcal) was assessed during each phase of the estrous cycle, for each diet, using a one-way ANOVA. Body weight gain was assessed using a between-subjects t-test. In Experiment 2, a 2×3 ANOVA was used to analyze differences in hypothalamic gene expression between diestrus (from Experiment 1) and OVX and OVX-PF rats consuming HFD or LFD. A 2×2 ANOVA was used to analyze differences in average daily food intake and body weight gain between OVX and OVX-PF. In Experiment 3, a 2×2 ANOVA was used to assess the effects of EB treatment on gene expression, average daily food intake and body weight gain in rats consuming HFD or LFD. Bonferroni post-hoc tests were used to compare groups when a significant main effect or interaction was detected. A significance level of $p < .05$ was used for all tests.

Results

Experiment 1: Influence of estrous cycle on hypothalamic gene expression

A significant interaction between diet and estrous phase was detected for prepro-QRFP mRNA in the VMH/ARC ($F = 4.0$, $p < .05$; Figure 1A). In HFD fed rats, prepro-QRFP mRNA level was highest during diestrus, while in the LFD fed rats, prepro-QRFP mRNA expression was highest during proestrus. A significant effect of diet on QRFP-r1 mRNA expression in the VMH/ARC was detected ($F = 4.3$, $p < .05$; Figure 1B). QRFP-r1 expression was highest during diestrus in HFD fed rats. No differences in prepro-QRFP mRNA in the LH were detected (Figure 1C), however QRFP-r2 mRNA was significantly affected by estrous cycle ($F = 4.8$, $p < .05$, Figure 1D), with the highest expression occurring during diestrus. Food intake differed across the estrous cycle in females consuming HFD ($F = 7.1$, $p < .01$) and LFD ($F = 6.8$, $p < .01$; Table 1). Rats in diestrus consumed more food than rats in estrus. Overall, rats consuming HFD gained more weight than rats fed LFD ($t = 5.6$, $p < .01$; Table 1).

Experiment 2: Effect of OVX on hypothalamic gene expression

Prepro-QRFP mRNA expression in the VMH/ARC was increased by HFD consumption ($F = 2.8$, $p < .05$) and by OVX ($F = 8.5$, $p < .01$; Figure 2A). Pairfeeding attenuated the OVX-induced increase in prepro-QRFP expression in HFD fed rats, but not in LFD fed rats. QRFP-r1 mRNA levels in the VMH/ARC were also increased by HFD consumption ($F = 5.6$, $p < .010$; Figure 2B). HFD and OVX did not affect prepro-QRFP or QRFP-r2 mRNA in the LH (Figure 2C, 2D). Average daily food intake was higher in the OVX rats, compared to OVX-PF rats ($F = 10.8$, $p < .01$; Table 1). Rats fed the HFD gained more weight than rats fed

the LFD ($F = 60.3$, $p < .01$) and OVX-PF gained less weight than OVX rats ($F = 10.3$, $p < .01$; Table 1).

Experiment 3: Hypothalamic gene expression following EB treatment

All rats received EB treatment every 4 days. Prepro-QRFP mRNA expression in the VMH/ARC was significantly reduced following EB treatment ($F = 20.3$, $p < .01$; Figure 3A). In the pre-EB group, prepro-QRFP mRNA was elevated following HFD consumption. EB treatment decreased QRFP-r1 mRNA expression ($F = 10.2$, $p < .01$; Figure 3B) in the VMH/ARC. No differences in prepro-QRFP mRNA expression in the LH were detected (Figure 3C), however EB treatment reduced QRFP-r2 expression in the LH ($F = 6.4$, $p < .05$; Figure 3D). Average daily food intake and body weight gain did not differ between the groups (Table 1).

Discussion

The neuropeptide, QRFP, is expressed in regions of the hypothalamus important in the regulation of feeding behavior (Arora and Anubhuti, 2006; Barsh and Schwartz, 2002; Bray, 1992; Chartrel et al., 2003; Clark et al., 1984; Fukusumi et al., 2006; Kageyama et al., 2012; Kampe et al., 2006; Qi et al., 2015; Schwartz et al., 2000). We have previously shown that in male and female rats, centrally administered QRFP-26 and QRFP-43 alters macronutrient selection and specifically increases the intake of a calorically dense HFD (Primeaux, 2011; Primeaux et al., 2013; Primeaux et al., 2008). Increases in the intake of HFD have been linked to an overconsumption of calories, which increases the propensity for weight gain, adiposity and subsequent risk of obesity. In rodent models, food intake fluctuates across the estrous cycle and many of the hypothalamic neuropeptides that regulate feeding behavior are influenced by estrous phase (Asarian and Geary, 2006, 2013; Eckel, 1999; Gao et al., 1997; Huang et al., 1993; Khorram et al., 1985; Leibowitz et al., 1998; Olofsson et al., 2009; Petersen et al., 1993; Santollo and Eckel, 2008a; Silva et al., 2010; Vigo et al., 2007a; Vigo et al., 2007b; Wade, 1972). The goal of the current series of experiments was to examine the effects of E2 on hypothalamic prepro-QRFP (VMH/ARC, LH), QRFP-r1 (VMH/ARC) and QRFP-r2 (LH) mRNA expression in female rats fed a HFD.

Experiment 1 investigated alterations in prepro-QRFP, QRFP-r1 and QRFP-r2 mRNA expression across the estrous cycle of rats consuming HFD or LFD. In HFD fed female rats, prepro-QRFP mRNA levels peaked during diestrus. Our data and others have shown that female rats consume more calories during diestrus than proestrus or estrus, with rats in estrus consuming the fewest calories (Asarian and Geary, 2006, 2013; Wade, 1972). Therefore, elevated QRFP expression in the VMH/ARC of HFD fed females during diestrus may lead to the increase in food intake associated with diestrus. The expression of QRFP-r1 in the VMH/ARC was also elevated during diestrus in HFD rats, suggesting a potential increase in the activity of QRFP in this brain region. In congruence with our previous report (Primeaux, 2011), prepro-QRFP mRNA expression in the VMH/ARC peaked during proestrus in LFD fed female rats, since proestrus is not associated with peak food intake across the cycle, QRFP may be playing a different role in LFD fed rats. Previous studies have shown that administration of QRFP-26 and QRFP-43 enhances basal and stimulated

luteinizing hormone secretion from the pituitary of cycling female rats (Navarro et al., 2006). In females, luteinizing hormone triggers ovulation. Therefore, an elevation in prepro-QRFP mRNA during proestrus in LFD fed rats may be important for stimulating luteinizing hormone release.

To further explore the relationship between E2 and hypothalamic QRFP and to investigate the role of dietary fat in this relationship, rats underwent OVX. The current study and others have reported that OVX induces hyperphagia and leads to increases in weight and adiposity (Eckel, 1999; Eckel and Geary, 1999; Eckel et al., 2000; Eckel and Moore, 2004; Majumdar et al., 2014; McElroy and Wade, 1987; Messina et al., 2006; Santollo and Eckel, 2008a; Schneider et al., 1986; Wade, 1975; Wade and Schneider, 1992). Therefore, pairfed groups (OVX-PF) were included in this study to control for hyperphagia. In this experiment, expression of prepro-QRFP mRNA following OVX was compared to the expression of rats in diestrus, since diestrus is associated with the lowest level of endogenous E2. As seen in Figure 2, prepro-QRFP expression in the VMH/ARC was elevated by OVX in rats fed the LFD. Pairfeeding did not alter this effect even though the OVX-PF rats consumed less food and gained less weight. However, in the HFD fed rats, OVX increased prepro-QRFP mRNA expression, which was attenuated by pairfeeding. Again, HFD intake and weight gain were decreased by pairfeeding and OVX-PF rats did not eat more HFD, than LFD. Prepro-QRFP expression in the VMH/ARC of the HFD fed OVX-PF group was elevated compared to the LFD fed rats in diestrus, suggesting that diet composition effects QRFP expression, in the absence of OVX-induced hyperphagia. Furthermore, despite pairfeeding, OVX-PF rats fed the HFD gained more weight than their LFD fed counterparts. These data suggest that both E2 and HFD influence QRFP expression and that a reduction in calories is not sufficient to reduce VMH/ARC expression of prepro-QRFP in the absence of E2. Following OVX, rats consuming the HFD expressed the highest levels of prepro-QRFP and QRFP-r1 mRNA, suggesting a potential increase in the activity of QRFP in this brain region.

The third experiment was designed to assess the direct role of E2 on hypothalamic QRFP, QRFP-r1 and QRFP-r2 mRNA expression. Following OVX, all rats underwent EB treatment every four days to mimic the rat estrous cycle. This protocol was used to prevent hyperphagia and excessive body weight gain following OVX. QRFP, QRFP-r1, and QRFP-r2 mRNA were measured pre-EB, which was the morning prior to EB treatment, and post-EB, which was the morning following EB treatment. These coincided with the lowest and highest morning levels of EB. EB treatment significantly decreased prepro-QRFP mRNA expression in the VMH/ARC of LFD and HFD fed rats, supporting the regulation of QRFP expression by E2. EB treatment also reduced the expression of QRFP-r1 and QRFP-2, particularly in the HFD rats, suggesting that EB is able to reduce the activity of QRFP. Similar to the findings in Experiment 2, HFD consumption increased QRFP expression in the VMH/ARC prior to EB treatment. Interestingly, this occurred without an observed increase in an overall average daily food intake or weight gain in the HFD fed rats, again suggesting an effect of dietary fat, which may not be dependent on hyperphagia and a potent effect of E2 on QRFP expression.

QRFP is a neuropeptide that has been conserved across species, suggesting that this neuropeptide has important implications for behavior. Previous studies from our laboratory

indicate that QRFP is a potent regulator of dietary fat intake in male and female rats (Primeaux, 2011; Primeaux et al., 2013; Primeaux et al., 2008). The current studies assessed the effects of E2 on hypothalamic QRFP, QRFP-r1 and QRFP-r2 expression in rats fed a HFD or a LFD. In cycling females, differential regulation of prepro-QRFP mRNA in the VMH/ARC by E2 was detected with HFD and LFD consumption, suggesting different roles of QRFP. The removal of endogenous E2 by OVX significantly elevated QRFP expression in the VMH/ARC and administration of EB significantly decreased QRFP expression in the VMH/ARC. These data support E2 as a direct regulator of QRFP's effects on dietary fat intake. Interestingly, prepro-QRFP mRNA expression in the LH was not affected by diet or E2, suggesting that this brain region is not an important modulator of QRFP's feeding effects, at least in our model. The mechanism by which QRFP alters food intake is not fully understood, though studies in males suggest that QRFP administration stimulates the expression and release of neuropeptide Y, while decreasing POMC expression and alpha-MSH release. Furthermore, neuropeptide Y1 and Y5 antagonists attenuate the effects of QRFP-26 on food intake (Lectez et al., 2009). We have reported that QRFP-induced HFD intake in males is attenuated by administration of a neuropeptide Y1 antagonist and a melanocortin agonist (Primeaux et al., 2013). E2 also regulates these neuropeptides; therefore, more studies are needed to fully understand the complex interaction between E2, QRFP and fat intake.

Acknowledgments

This research was supported by LSUHSC-NO to SDP. This work was supported in part by P20-RR021945 from the National Center for Research Resources and NIH Center Grant 1P30 DK072476 to Pennington Biomedical Research Center.

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Highlights

- prepro-QRFP mRNA expression in the VMH/ARC is affected by estrous phase and HFD.
- Ovariectomy and HFD intake increase prepro-QRFP mRNA levels in the VMH/ARC.
- Estradiol Benzoate administration decreases prepro-QRFP mRNA levels in the VMH/ARC.
- Estradiol regulates the expression of QRFP in the VMH/ARC of female rats.

Figure 1.

Estrous phase was determined in rats consuming HFD or LFD. **A.** In the VMH/ARC, prepro-QRFP mRNA expression was elevated during proestrus in LFD fed rats and during diestrus in HFD fed rats. **B.** QRFP-r1 expression was increased in the VMH/ARC during diestrus of HFD fed rats. **C.** Prepro-QRFP was not altered in the LH. **D.** QRFP-r2 mRNA levels in the LH were increased during diestrus in HFD fed rats. Values are shown relative to cyclophilin levels and as a fold-change from LFD fed rats in diestrus. Data is shown as mean $\pm$ SEM, $p < .05$. * within diet; + across diet.

Figure 2.

A. Consumption of HFD and OVX increased prepro-QRFP mRNA expression in the VMH/ARC. Pairfeeding attenuated OVX-induced increases in prepro-QRFP mRNA level in HFD fed rats. **B.** HFD intake following OVX increased the expression of QRFP-r1 in the VMH/ARC. **C.** Prepro-QRFP mRNA expression in the LH was not altered by HFD intake or OVX. **D.** QRFP-r2 expression in the LH was not affected by HFD or OVX. Values are shown relative to cyclophilin levels and as a fold-change from LFD fed rats in diestrus. Data is shown as mean $\pm$ SEM, $p < .05$. * within diet vs. diestrus; + across diet

Figure 3.

Following OVX, all rats received EB every 4 days. Based on the EB administration schedule, rats in the pre-EB group had the lowest levels of estradiol and rats in the EB group had the highest levels of estradiol. **A.** HFD intake increased prepro-QRFP mRNA in the VMH/ARC of rats in the pre-EB group. EB treatment reduced prepro-QRFP levels in all rats. **B.** EB treatment decreased QRFP-r1 mRNA expression in the VMH/ARC. **C.** Prepro-QRFP mRNA expression in the LH was not altered by EB treatment. **D.** EB treatment decreased QRFP-r2 expression in the LH. Values are shown relative to cyclophilin levels and

as a fold-change from LFD fed rats in diestrus. Data is shown as mean $\pm$ SEM, $p < .05$. * within diet; + across diet.

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Table 1

Body weight gain and average daily food intake from Experiments 1–3.

	Body Weight Gain (g)		Average Daily Food Intake (kcal)	
	Low Fat Diet	High Fat Diet	Low Fat Diet	High Fat Diet
Experiment 1: Estrous Cycle				
Diestrus	41.3 ± 2.5	63.4 ± 3.1 ⁺	61.3 ± 1.4	71.9 ± 2.3
Proestrus			58.9 ± 2.5	68.4 ± 2.5 [*]
Estrus			51.5 ± 2.3 [*]	59.6 ± 1.9 [*]
Experiment 2: OVX				
OVX	70.8 ± 4.4	109.4 ± 7.1 ⁺	78.6 ± 3.5	86.8 ± 4.4
OVX-Pair Fed	51.1 ± 3.5 [*]	89.0 ± 2.7 ⁺ [*]	61.5 ± 0.6 [*]	68.8 ± 0.8 [*]
Experiment 3: EB treatment				
EB treated (all)	50.3 ± 4.6	45.1 ± 3.4	59.1 ± 1.5	57.7 ± 1.1

Exp. 1: + p<.05 vs. LFD; *p<.05 vs. diestrus

Exp. 2: + p<.05 vs. LFD; *p<.05 vs. OVX-PF

Exp. 3: n.s. p>.05