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Female mice and rats exhibit species-specific metabolic and behavioral responses to ovariectomy

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Abstract

Ovariectomy (OVX) leads to hyperphagia and weight gain in rats, which can be prevented by estradiol (E2) replacement; however, the role of endogenous E2 on feeding and energy homeostasis in female mice has not been well characterized. The primary goal of this study was to assess the relative contribution of increased energy intake and decreased energy expenditure to OVX-induced weight gain in female rats and mice. OVX led to hyperphagia in rats, but did not produce daily, nor cumulative, hyperphagia in mice. OVX decreased mass-specific metabolic rate in mice, but not in rats. OVX decreased home cage locomotor activity in both species. Pair-feeding attenuated OVX-induced weight gain in rats and produced both short- and long-term changes in expression of key hypothalamic genes involved in food intake and energy homeostasis, i.e., the anorexigenic neuropeptide pro-opiomelanocortin (POMC) and the orexigenic neuropeptides: melanin-concentrating hormone (MCH) and agouti-related peptide (AgRP). No differences in hypothalamic gene expression were observed between OVX'd and sham mice. The results suggest that OVX-induced weight gain is mediated by hyperphagia and reduced locomotor activity in rats, but that in mice, it is primarily mediated by reduced locomotor activity and metabolic rate.

Keywords

energy expenditure; locomotor activity; indirect calorimetry; food intake; RT-PCR; hypothalamic neuropeptides

1. Introduction

There is uncertainty regarding the evidence that estradiol (E2) influences energy homeostasis by regulating energy expenditure. Pair-feeding of ovariectomized (OVX'd) rats generally does not prevent weight gain (Roy and Wade, 1977); however, this finding is not universal (Liang et al., 2002). If OVX-induced weight gain is not prevented by restricting daily food intake of OVX'd rats, then a role for E2 in the regulation of energy expenditure is strongly suggested. Thus, the primary goal of this study was to assess the relative

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contribution of increased energy intake and decreased energy expenditure to OVX-induced weight gain in female mice and rats.

In addition, it is well established that E2 exerts both a tonic and a phasic inhibitory effect on food intake in female rats (reviewed in Eckel, 2004). While the hypophagia and hyperactivity that occur on estrus have been well characterized in the female rat (Wade, 1972) they have not been well characterized in the female mouse. In fact, *exogenous* E2 treatment has been found to increase locomotor activity in OVX'd female mice (Ogawa et al., 2003), yet surprisingly few studies have investigated the role of *endogenous* E2 on food intake and locomotor activity in the ovarian-intact female mouse. Of these studies, the following conclusive statements can be made: 1.) an estrous rhythm of locomotor activity has not been established in ovarian-intact female mice and 2.) an estrous-related decrease in food intake has not been observed in ovarian-intact female mice (in fact, the absence of these effects has been reported (Barnett and McEwan, 1973; Petersen, 1976)). In light of the increasing use of mice as the pre-clinical vertebrate model of choice, it is crucial to know how exactly endogenous E2 affects food intake and energy homeostasis in the normal ovarian-intact mouse. Experience tells us that we cannot simply extrapolate from what is already known about E2 in the ovarian-intact rat to the mouse. Furthermore, evidence involving exogenous, pharmacological doses of E2 does not necessarily indicate how endogenous, physiological levels of E2 affect the physiology of the ovarian-intact mouse. Therefore, the secondary goal of this study was to characterize the daily food intake and locomotor activity patterns of ovarian-intact mice. After observing hyperphagia in OVX'd rats (an effect that was clearly absent in OVX'd mice), we sought to measure changes in gene expression of key hypothalamic neuropeptides involved in feeding and energy homeostasis.

2. Materials and Methods

2.1 Animals and housing

Female C57BL/6J mice (14 wk old) and Long-Evans rats (14 wk old) were obtained from Charles River Laboratories (Charlotte, NC). Upon arrival, animals were housed individually in polycarbonate cages containing wood chip bedding and a red, translucent igloo niche (Bio-Serv, Frenchtown, NJ; niches were in mouse cages only). Animals were given 2 wk to fully recover from shipment stress and to acclimate to a reversed circadian cycle (11a-11p dark:11p-11a light). Pellet chow (4.5% fat, physiological caloric value = 3.3 kcal/g; Purina 5001; Purina Ralston, Richmond, IN) and deionized water were made freely available. The room was maintained at 23 ± 1.0°C. Animal usage and all procedures were in strict compliance with the guidelines of the National Institutes of Health and approved by the Florida State University Institutional Animal Care and Use Committee.

2.2 Indirect calorimetry and behavioral monitoring

Measurements of gas exchange and monitoring of animal behavior were performed using previously published approaches (Rashotte et al., 1995). At ~ 4 mos of age, animals were housed in individual cages fitted with custom-made polycarbonate lids providing a near air-tight seal for continuous determination of 23-h oxygen consumption (VO_2; ml/min) and carbon dioxide production (VCO_2; ml/min). Respiratory quotient (RQ; VCO_2/ VO_2), an index of substrate utilization, was calculated from these measures. Total energy expenditure was estimated using the Weir equation: energy expenditure = (kcal/min) = $3.91(VO_2)$ + $1.1(VCO_2)/1{,}000$ (Simonson and DeFronzo, 1990). VO_2 was normalized to the recorded daily body weight of each animal. Energy balance was calculated using the value for total energy expenditure and subtracting it from the recorded daily caloric intake. Gas-exchange data were obtained for 23 h. During the excluded 1 h (10a-11a) of the full 24 h day, animals

were alert and handled (see below); thus the equivalent of an additional 1 h of average dark-phase energy expenditure was added to estimate total daily energy expenditure.

The cage calorimeters were housed inside environmental chambers to provide more precise control of cage ambient temperature (± 0.1°C). The shoebox cage was positioned on a custom-designed force platform sensitive to the transfer of the animal's mass throughout the cage in order to obtain quantification of locomotor activity. While housed in the cage calorimeters, animals were given free access to a powdered form of the same pellet chow described above. Food intake, water intake and body weight of each animal were determined during a daily maintenance period that occurred 1 h before the onset of dark. At this time, daily behavioral and metabolic data were compiled and transferred for offline processing.

2.3 Acclimation, baseline, and ovariectomy (OVX) or sham surgery

Animals were allowed to acclimate to the metabolic housing units for ~ 6-7 d. Cycle phase was determined daily during the last hour of the light phase by cellular profile analysis of vaginal mucosa smear samples (taken by lavage). Standard criteria (Becker et al., 2005) were used to assign cycle phase, according to the types of cells present in the samples as determined by observing them under a light microscope. Once acclimated, 3 d of baseline data were collected for later comparison to data collected post-surgery. On the last day of baseline, animals were anesthetized with halothane and were either ovariectomized (OVX'd, n = 10/mice; n = 12/rat) or they underwent sham surgery (n = 10/mice; n = 8/rat). The intra-abdominal OVX surgery consisted of exposing the ovaries via a single midline skin incision. Each ovary was cauterized and excised at the tip of the uterine horn. The retroperitoneal muscle was then re-closed with silk suture and skin was re-closed with wound clips.

To compare responses to OVX, mice (n = 10 sham, n = 10 OVX) and rats (n = 8 sham, n = 12 OVX) were returned to their home cage calorimeters after surgery and observed daily for 3 wk. After 3 wk, all animals were euthanized (shams were terminated during estrus) with an overdose of pentobarbital sodium (60 mg/kg i.p.; Nembutal Sodium Solution, Henry Schein, Inc., Melville, NY). Animals were decapitated and hypothalami were immediately removed and placed in liquid nitrogen, and then stored at -80°C. Fat pads were removed and weighed.

2.4 Locomotor activity and food intake in ovarian-intact female mice

In order to determine the role of *endogenous* estradiol in regulating locomotor activity and food intake in the ovarian-intact mouse, an additional subset of ovarian-intact female C57/B6 mice (4 mos old, n = 8) were obtained and housed in home cage calorimeters for ~ 30 d. Cycle phase was determined daily (as described above in *2.3.*).

2.5 Effect of pair-feeding on OVX-induced weight gain

Two additional cohorts of Long-Evans rats (14 wks of age) were obtained. The use of the home cage calorimeters was not employed for this additional experiment. To determine the role of food intake on OVX-induced weight gain, these two subsets of rats were part of either a "short-term" (3 wk) or a "long-term" (13 wk) pair-feeding study. In both pair-feeding studies, OVX'd rats were given either *ad libitum* access to powdered chow (OVX-AL, n = 4/per study) or they were individually pair-fed each day to the amount of food consumed daily on non-estrous days prior to OVX (OVX-PF, n = 5/per study). By preventing the OVX'd rats from over-eating, pair-feeding allows for the factor of increased food intake to be excluded when observing the effect of OVX on body weight. Sham rats (n = 4/per study) were given *ad libitum* access to powdered chow and were terminated on estrus at the end of the study.

2.6 Measurement of hypothalamic gene expression using reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from whole hypothalamic tissue using TRIzol reagent (Invitrogen) in conjunction with phase lock gels (Eppendorf). Each sample received chloroform, was shaken vigorously for 15 sec, and then left to stand at room temperature for 3 min. Total RNA was precipitated from all samples. Concentrations of each RNA sample was obtained using a NanoDrop Spectrophotometer (ND-1000, Thermo Fisher Scientific) and its integrity was determined by denaturing gel electrophoresis as previously described (Goda and Minton, 1995). cDNA synthesis was performed by using iScript cDNA Synthesis Kit (Bio-Rad). RT-PCR was performed using the iCycler iQ Real-Time PCR Detection System (BioRad) in conjunction with iQ SYBR Green Supermix (BioRad). All data were normalized to the expression of the housekeeping gene RP27, as previously described (Resuehr et al., 2006). The PCR cycling conditions were as follows: HotStart 5 min at 95°C, cycling 40 times at 95°C denaturation for 15 s, annealing and fluorescent data collection at 55°C for 30 sec, extension at 72°C for 30 sec. A melt curve was performed to assess the stringency of the PCR after the cycling. A negative control was performed by omission of template cDNA. The primers used in this study (Table 1) were for some of the key hypothalamic neuropeptides involved in food intake and energy homeostasis, i.e., the anorexigenic neuropeptides: pro-opiomelanocortin (POMC) and leptin (ObRb) and the orexigenic neuropeptides: melanin-concentrating hormone (prepro-MCH), MCH receptor-1 (MCHR-1), neuropeptide-Y (NPY), agouti-related peptide (AgRP), and orexin (ORX).

2.8 Statistical analyses

Using SigmaPlot 12.0 statistical software, mean differences were analyzed using two-way repeated measures ANOVA (repeated measure = time (days) x treatment group) to evaluate effect of treatment (e.g., SHAM vs. OVX) over time for dependent variables (i.e., body weight, food intake, locomotor activity, VO_2). Tukey's post-hoc tests were used to determine specific days on which treatment groups were significantly different from one another. Single-value measurements, e.g., effect of OVX or pair-feeding on hypothalamic gene expression, were statistically assessed by one-way ANOVA. Tukey's post hoc test was used to investigate differences between means after significant ($p < 0.05$) main or interactive ANOVA effects were found.

3. Results

3.1 Energy homeostasis and ingestive behavior

Mice—OVX'd mice gained weight (Fig.1*A*) with no accompanying increase in food intake; neither daily (Fig.1*B*), nor cumulative (shams = 93.4 +/- 6.6 g, OVX = 96.6 +/- 4.9 g; post-surgical days 1-21) food intake increases were observed in OVX'd mice. Other than on post-surgical days 1 and 2, daily energy expenditure and daily energy balance did not differ between OVX and sham mice (Fig.1C and *D*. respectively, $p > 0.05$).

Ovarian-intact mice (n = 8) exhibited no difference in daily food intake on estrous- vs. non-estrous days (5.5 ± 0.4 g vs. 5.6 ± 0.3 g) over a period of 21 d.

Rats—OVX'd rats gained weight (Fig. 2*A*) with an accompanying increase in daily food intake (Fig. 2*B*). Daily energy expenditure was greater in OVX'd rats, compared to their sham counterparts (Fig. 2*C*, $p < 0.05$). Daily energy balance was greater in OVX'd rats, compared to their sham counterparts (Fig. 2*D*, $p < 0.05$).

3.2 Oxygen consumption (VO_2), respiratory quotient (RQ), and locomotor activity

Compared to baseline, OVX'd mice (Fig. 3*A*, $p < 0.05$), but not rats (Fig. 3*C*, $p > 0.05$), exhibited decreased dark-phase mass specific oxygen consumption (VO_2). Light-phase mass specific metabolic rate was not different between groups in either species (data not shown). Indicating a shift in substrate utilization toward fat storage, both OVX'd rats and mice had greater respiratory quotient (RQ) values compared to their sham counterparts ($p < 0.05$, both dark- and light-phase RQ were different in rats: OVX dark RQ = 0.94 ± 0.01, sham dark RQ = 0.91 ± 0.01, OVX light RQ = 0.93 ± 0.01, sham light RQ = 0.90 ± 0.01; light-phase, but not dark-phase, RQ was different in mice, OVX light RQ = 0.89 ± 0.01 sham light RQ = 0.86 ± 0.01).

OVX decreased dark-phase locomotor activity in both mice (Fig.3*B*) and rats (Fig.3*D*), compared to their respective baselines and compared to the dark-phase locomotor activity of their sham counterparts (in mice only). Light-phase locomotor activity was not different between groups in either species ($p > 0.05$, data not shown).

Ovarian-intact mice exhibited no difference in dark-phase locomotor activity on estrous- vs. non-estrous days (361 ± 16 m vs. 379 ± 14 m) over a period of 21 d (post-acclimation to the home cage calorimeters).

3.3 Fat pads

OVX increased parametrial fat in both species (Fig.4*A*, *Mice*: degrees of freedom (d.f.) = 19, F = 6.94, $p = 0.017$; *Rats*: d.f. = 23, F = 6.12, $p = 0.022$), compared to shams. OVX increased mesenteric fat in rats (Fig.4*B.2*., F = 15.8, $p < 0.001$), but not in mice (Fig.4*B.1*., $p > 0.05$). OVX increased retroperitoneal fat in mice (Fig.4*C.1*, F = 5.43, $p < 0.05$), but not in rats (Fig.4*C.2*., F = 4.23, $p = 0.053$).

3.4 Pair-feeding

Pair-feeding attenuated, but did not prevent, OVX-induced weight gain (*short-term pair-feeding cohort*: 3 wk weight gain: SHAM = 10.2 +/- 8.5, OVX-pair-fed = 28.5 +/- 8.5, OVX-*ad libitum*-fed = 51 +/- 10.5 g). By post-surgical week 5, the pair-fed group had developed a clear intermediate body weight that was greater than that of shams (Fig.5*A*, d.f. = 12, F = 23.6, $p = 0.007$), yet less than that of OVX *ad libitum*-fed rats (Fig.5*A*, $p = 0.022$). This spread of body weights (OVX-AL > OVX-PF > SHAM) remained consistent throughout the remainder of the 13-wk study, despite the variability in food intake for the OVX-AL and sham rats from week to week (Fig.5*B*).

3.5 Hypothalamic mRNA expression

Rats—Compared to *ad libitum*-fed OVX'd rats, both short-term (3.5 wk) and long-term (13 wk) pair-feeding of OVX'd rats led to a reduction in mRNA of the orexigenic neuropeptide, prepro-MCH (Fig.6*A*, $p < 0.05$, d.f. = 12, short term: F = 3.55, $p = 0.048$; Fig.6*B*, long-term: F = 8.64, $p = 0.009$ OVX-AL vs. OVX-PF; $p = 0.02$ OVX-AL vs. SHAM). Short-term pair-feeding led to a reduction in the anorexigenic neuropeptide, POMC mRNA in OVX rats (Fig.6*B*, d.f. = 12, F = 5.23, $p = 0.01$ OVX-AL vs. OVX-PF). Correspondingly, sham rats had less POMC mRNA expression than OVX rats (Fig.6*B*, d.f. = 12, F = 5.23, $p = 0.02$ OVX-AL vs. SHAM). OVX'd rats (both PF and AL) in the long-term study had reduced AgRP levels, compared to shams (Fig.6*C*, d.f. = 12, F = 6.89, $p = 0.01$ SHAM vs. OVX-AL; $p = 0.01$ SHAM vs. OVX-PF). By 13 wk, all rats (OVX-PF, OVX-AL, and shams) had enhanced AgRP mRNA expression, compared to the rats in the short-term PF cohort (terminated at 3.5 wk post-surgery; Fig.6*C*, $p = 0.009$). There were no differences found in

NPY, ORX, or ObRb mRNA expression (data not shown, $p > 0.05$) in the short-term study; these genes were not quantified in the long-term study.

Mice—In an additional cohort of mice (N=4/group, we determined the effect of OVX on expression of three key hypothalamic genes involved in feeding (prepro-MCH, MCHR-1, and POMC mRNA). Because no changes in food intake were observed between OVX'd and sham mice (Fig.1*B*, $p > 0.05$), no changes were expected in orexigenic and/or anorexigenic tone. Consistent with the behavioral data indicating a lack of hyperphagia in OVX'd mice, no changes in hypothalamic gene expression was found between OVX'd and sham mice in any of these genes (data not shown; prepro-MCH: d.f. = 7, F = 0.22, $p = 0.66$; MCHR-1: F = 1.513, $p = 0.24$; POMC: F = 1.23, $p = 0.29$).

4. Discussion

The primary goal of this study was to assess the relative contribution of energy intake and expenditure to OVX-induced weight gain in female mice and rats. Three new findings were obtained. First, OVX-induced weight gain in rats is mediated by hyperphagia and reduced locomotor activity; while in mice, it is mediated by reduced locomotor activity and reduced metabolic rate. Second, pair-feeding of OVX'd rats attenuated, but did not prevent, OVX-induced weight gain. Lastly, ovarian-intact mice did not demonstrate estrous-related hypophagia, nor home cage hyperactivity.

To date, the role of endogenous E2 on food intake and locomotor activity in the ovarian-intact mouse has not been well characterized. Of the few studies that have measured these parameters in ovarian-intact mice, the absence of both an estrous-related increase in locomotor activity and decrease in food intake have been reported (Barnett and McEwan, 1973; Petersen, 1976; Meziane et al., 2007). The results of the present study are consistent with these findings, suggesting that the *phasic* effect of E2, while very well established in the rat, is notably absent in the mouse. To our knowledge, no other studies have measured food intake and/or home cage activity across the estrous cycle in ovarian-intact mice. This may be due, at least in part, to the inherent difficulty in assessing the murine estrous cycle, as mice tend to have longer cycles than rats (~ 7 d vs. ~ 4 d) and are prone to atypical cycling patterns (vom Saal et al., 1994).

With regard to the *tonic* effect of E2 on food intake in mice, the evidence is conflicting. One study reports a cumulative increase over time in OVX'd mice (Geary et al., 2001), an effect that was unexpectedly absent in the present study. The lack of corroboration could be due to: different ages and strains of mice and different length of time of post-OVX observation. In contrast, another study reports that OVX'd vehicle-treated mice do not exhibit hyperphagia, but do exhibit increased post-OVX weight gain (Blaustein et al., 1976), which is consistent with the present study. Additionally, a third group reports that at 4 mos post-OVX, there was no difference in food intake between sham and OVX'd mice, but that by 5 mos, OVX'd mice ate slightly more (Gomori et al., 2007). These findings support the notion that, unlike rats, 3 wk post-OVX is not a sufficient amount of time to observe a food intake effect in mice. Finally, exogenous E2 injections in OVX'd mice have been shown to produce a decrease in food intake (Blaustein et al., 1976), yet they were unable to decrease body weight, suggesting that OVX-induced body weight and food intake changes may be dissociable. Taken together, it is possible that *endogenous* E2 may not be a potent regulator of food intake in the female mouse. However, the possibility that endogenous E2 modulates micro-meals and that the post-OVX observation period must exceed 4 wk in order to observe a food intake effect cannot be excluded.

In contrast to mice, it is well established that E2 has a phasic (Eckel et al., 2000) and tonic (Geary and Asarian, 1999) inhibitory effect on feeding in female rats. The present study confirms that OVX'd rats are hyperphagic, have greater energy expenditure, and are in greater positive energy balance than their sham counterparts. Furthermore, female rats are hyperactive on estrus (Finger, 1969) and E2 replacement in OVX'd rats increases activity (Gentry and Wade, 1976), while OVX'd rats with no E2 replacement are hypoactive (Wade, 1972). OVX-induced hypoactivity has also been reported in mice (Heine et al., 2000; Gorzek et al., 2007). Consistent with these findings, both mass-specific metabolic rate and locomotor activity were suppressed in OVX'd mice in the present study, while only locomotor activity was suppressed in OVX'd rats. It is reasonable to suggest that instead of suppressing food intake, endogenous E2 may act mainly by modulating these components of energy homeostasis (metabolic rate and locomotor activity) in female mice since OVX'd mice were not hyperphagic, yet still gained weight rapidly.

The observation that energy expenditure was not suppressed in OVX'd animals of either species, is perplexing, yet it is not a new finding. Some have sought to understand this by looking at fuel utilization (Ainslie et al., 2001; Chen and Heiman, 2001), intrascapular brown adipose tissue (iBAT) weight (Ainslie et al., 2001), iBAT protein content (Richard, 1986), and the amount of bound guanosine diphosphate (GDP) (Richard, 1986). While no differences have been reported between OVX'd and sham *rats*, to our knowledge, these parameters have not been reported in *mice*. Such information may provide additional insight into why OVX'd mice get obese, despite normophagia and unchanged energy expenditure. Nonetheless, it is important to acknowledge the possibility that our indirect calorimetry system may not be sensitive enough to measure the small changes in oxygen consumption that likely occur in the relatively small mouse.

Because of conflicting reports regarding the contribution of increased food intake to OVX-induced weight gain in the rat ((Roy and Wade, 1977; Shimomura et al., 1989) vs. (Liang et al., 2002)), we performed short- and long-term pair-feeding experiments. The data show that pair-feeding attenuated, but did not prevent, OVX-induced weight gain. This pivotal finding strongly suggests a role for E2 in regulating energy expenditure, as decreased food intake alone did not prevent OVX-induced weight gain.

To investigate whether key hypothalamic neuropeptides involved in energy homeostasis are altered by lack of ovarian hormones, we measured total hypothalamic mRNA levels of a number of genes that have been linked to having a role in E2-induced hypophagia. E2 decreases the orexigenic effect of MCH (Messina et al., 2006). Compared to *ad libitum*-fed OVX'd rats, pair-fed OVX'd rats had reduced total hypothalamic prepro-MCH mRNA. The lack of an increase in prepro-MCH mRNA in pair-fed rats was at first perplexing, but seems reasonable when considering the fact that these rats were not fasted, but were merely pair-fed. It is also possible that the metabolic adaptations associated with obesity have overridden the normal signals that would have otherwise increased prepro-MCH in response to mild caloric restriction.

Given that MCH neurons are likely targets of E2 action (Murray et al., 2000; Mystkowski et al., 2000), it was predicted that endogenous E2 would exert an inhibitory effect on prepro-MCH mRNA expression. Thus, it was surprising that prepro-MCH mRNA levels of shams (terminated on estrus) were not decreased. That an effect was not observed could be due to temporal compensation by other neuropeptides involved in energy homeostasis, or because an optimal inhibitory effect of endogenous E2 on prepro-MCH mRNA would be best uncovered at a different time point, i.e., at proestrus, when circulating E2 levels are highest. Such critical factors require further experimental investigation.

Consistent with previous reports (Brady et al., 1990; Kim et al., 1996), short-term pair-feeding led to a reduction in POMC mRNA, supporting the notion of POMC being a critical catabolic effector (Schwartz et al., 2003). That shams did not exhibit any long-term alterations in POMC mRNA compared to OVX'd rats is consistent with previous reports finding no phasic changes in POMC mRNA in ovarian-intact rats (Rocha et al., 2004), in OVX'd rats treated with E2 (Treiser and Wardlaw, 1992), nor in OVX'd vs. ovarian-intact rats (Wise et al., 1990). Also consistent with previous reports (Korner et al., 2001; Rocha et al., 2004; Santollo and Eckel, 2008), there were no differences in AgRP mRNA between any of the groups at the 3.5 wk time point. However, the presence of endogenous E2 (in shams) was associated with increased AgRP mRNA at the 13.5 wk time point. This is consistent with the observed increase in AgRP mRNA reported in a model of chronic hyperestrogenemia in male rats (Mystkowski et al., 2000), suggesting the possibility that increased AgRP mRNA occurs in response to estrous-related hypophagia.

Consistent with the behavioral data indicating a lack of hyperphagia in OVX'd mice, no changes in hypothalamic gene expression were observed between OVX'd and sham mice, suggesting there were no alterations in orexigenic nor anorexigenic tone, as measured by these genes. Whether or not other genes involved in energy homeostasis are implicated in the physiology of the OVX'd mouse remains to be elucidated.

In conclusion, the results indicate that clear species-specific differences exist between rats and mice in response to OVX. Namely, OVX-induced weight gain is mediated by hyperphagia and hypoactivity in rats, but it is largely mediated by hypoactivity and reduced metabolic rate in mice. The possibility that altered substrate utilization plays a role in OVX-induced obesity and the possibility of nuclei-specific changes in hypothalamic mRNA expression warrant further investigation. Lastly, pair-feeding of OVX'd rats attenuates, but does not prevent OVX-induced weight gain, strongly suggesting a metabolic role for E2-modulation of energy homeostasis.

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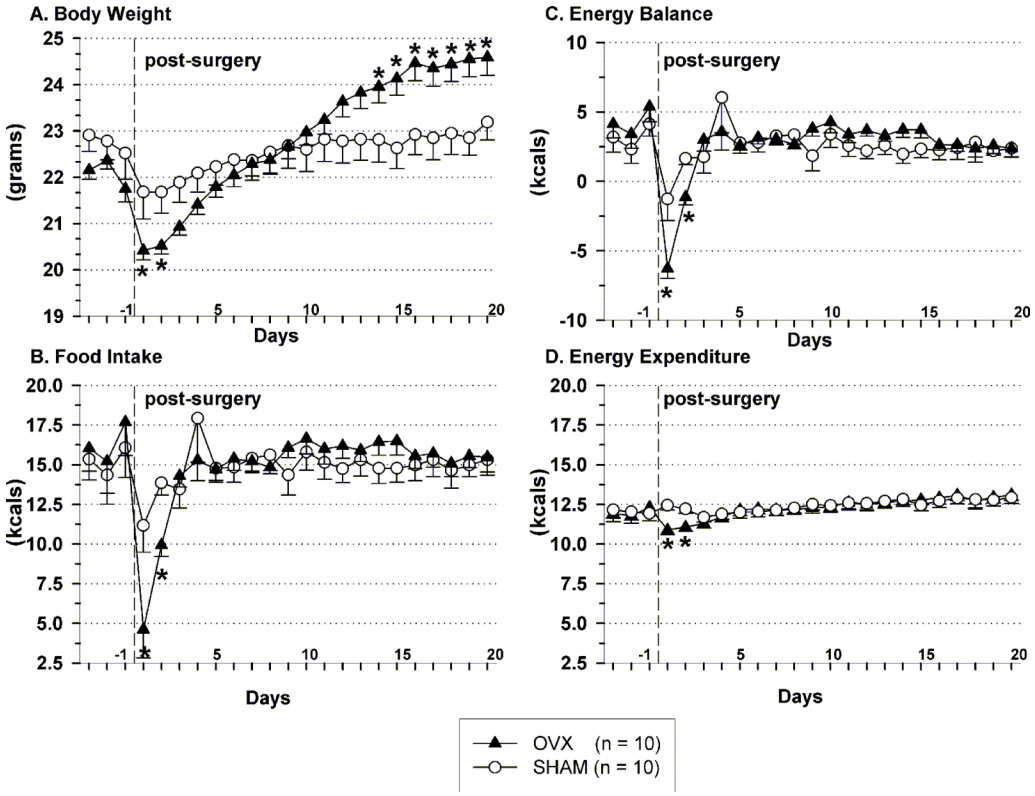

Fig. 1.

Body weight (*A*), daily caloric intake (*B*), energy balance (*C*) and energy expenditure (*D*) during 3 baseline and 20 post-surgical days in ovariectomized (triangles) and sham (circles) mice. The vertical dashed line indicates the end of the baseline period and the day when surgery was performed. Data represent mean ± SEM, n = 10/per group; (*) indicates $p < 0.05$ for OVX vs. SHAM mice and for pre-OVX baseline vs. post-OVX data. *Abbreviation*: OVX, ovariectomized.

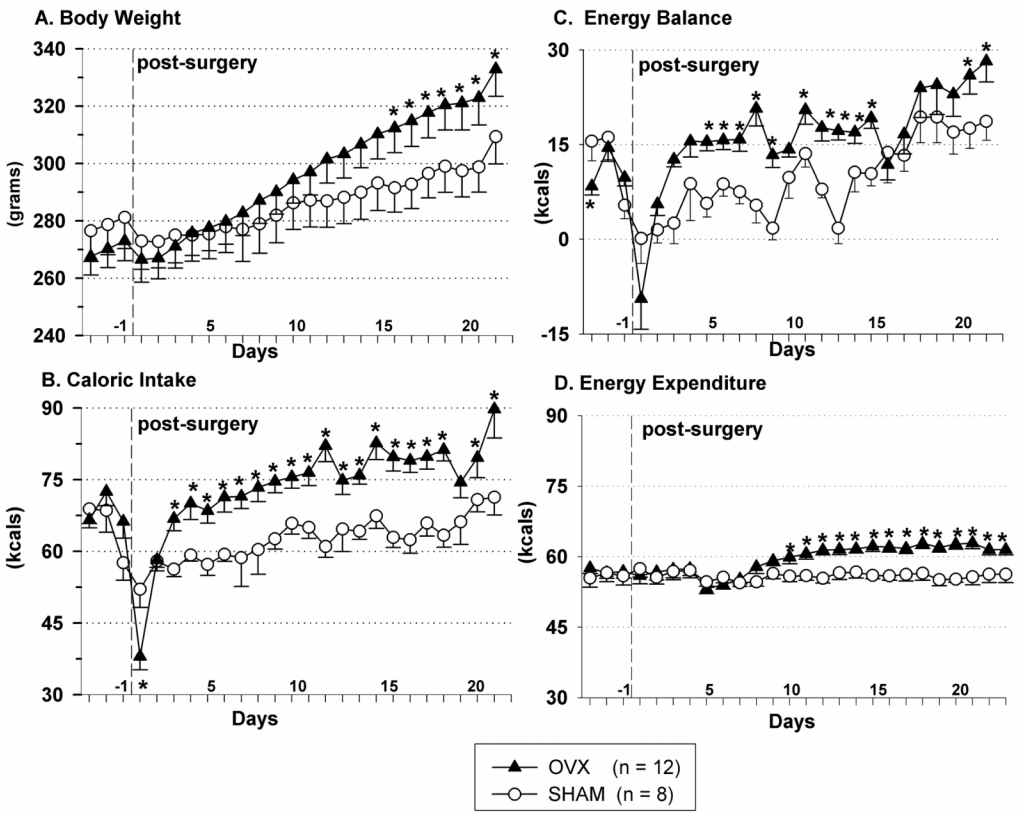

Fig. 2.

Body weight (*A*), daily caloric intake (*B*), energy balance (*C*) and energy expenditure (*D*) during 3 baseline days and 22 post-surgical days in ovariectomized (triangles) and sham (circles) rats. The vertical dashed line indicates the end of the baseline period and the day when surgery was performed. Data represent mean ± SEM, n = 12/OVX, n = 8/SHAM; (*) indicates $p < 0.05$ for OVX vs. SHAM rats. *Abbreviation*: OVX, ovariectomized.

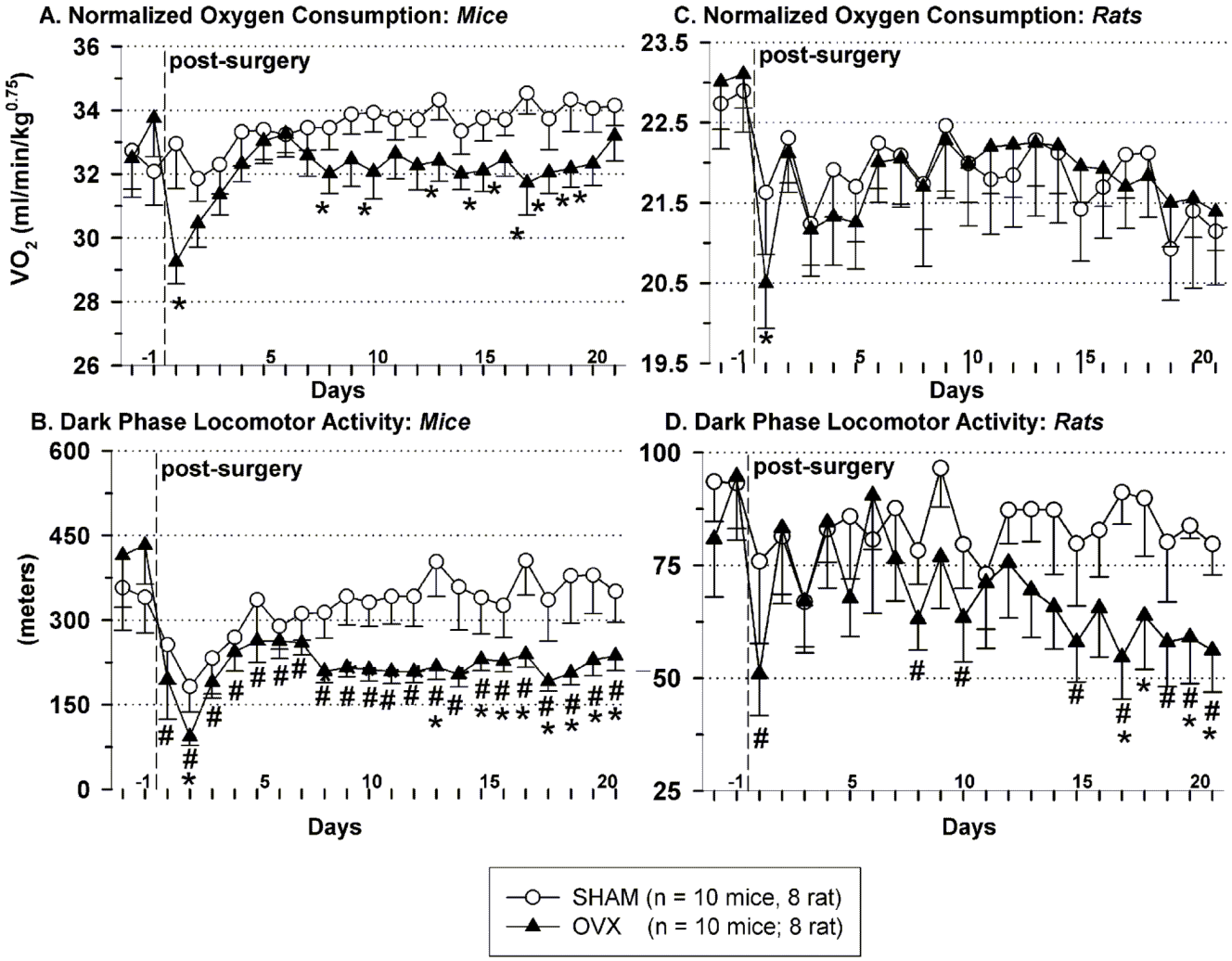

Fig. 3.

Dark-phase oxygen consumption (VO_2), normalized to body weight, in mice (*A*) and rats (*C*). Dark-phase locomotor activity in mice (*B*) and rats (*D*). The vertical dashed line indicates the end of the baseline period and the day when surgery was performed. Data represent mean ± SEM; mice: n = 10/per group, rats: n = 8/per group; (*) indicates $p < 0.05$ for OVX vs. sham animals; (#) indicates $p < 0.05$ for pre-OVX baseline vs. post-OVX data. *Abbreviation*: OVX, ovariectomized. *Note: different scales are used for mouse vs. rat data.

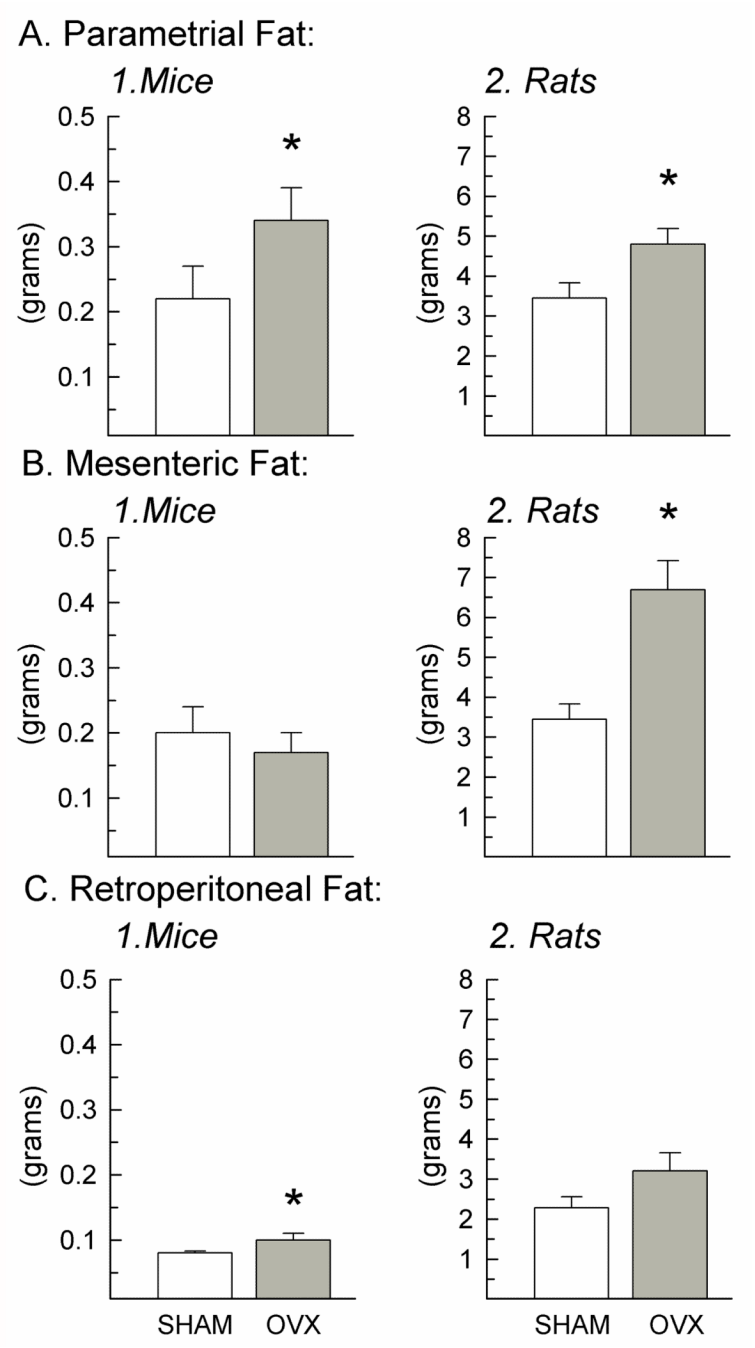

Fig. 4.

Parametrial, mesenteric, and retroperitoneal fat pad weights excised at the termination of the study of OVX (gray bars) and sham (white bars) mice (*A1, B1, C1*; n = 10/group) and rats (*A2, B2, C2*; n = 12/group). Data represent mean ± SEM; (*) indicates $p < 0.05$ in OVX vs. sham animals. *Abbreviation*: OVX, ovariectomized.

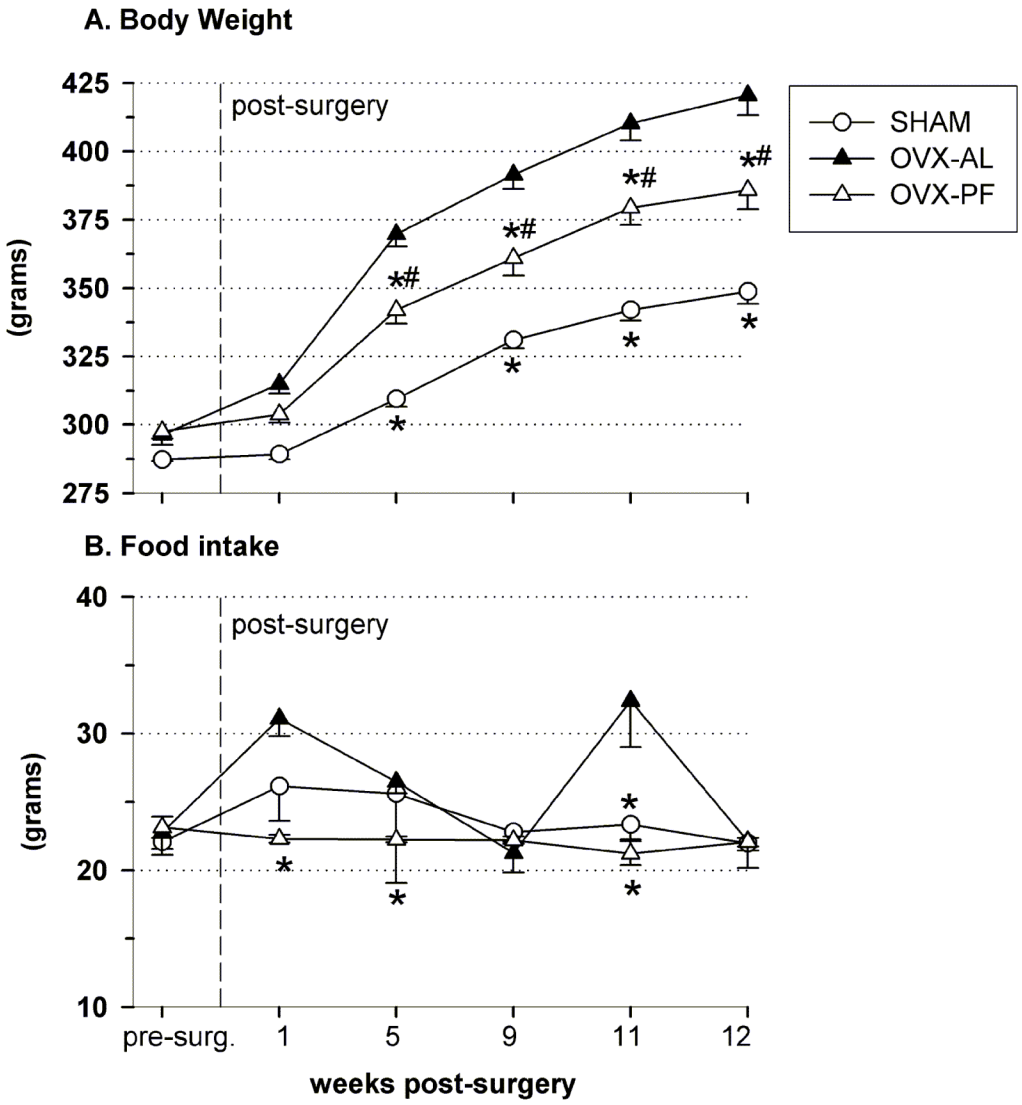

Fig. 5.

Body weight (*A*) and food intake (*B*) of OVX-AL (n = 4; closed triangles), OVX-PF (n = 5; open triangles), and SHAM (n = 4; circles) rats in the long-term pair-feeding study. Food intake values are 4-day averages; sham values represent food intake data obtained on non-estrous days only. Data represent mean ± SEM; (*) indicates $p < 0.05$ compared to OVX-AL, (#) indicates $p < 0.05$ compared to SHAM. *Abbreviations*: OVX, ovariectomized; AL, *ad libitum*-fed; PF, pair-fed.

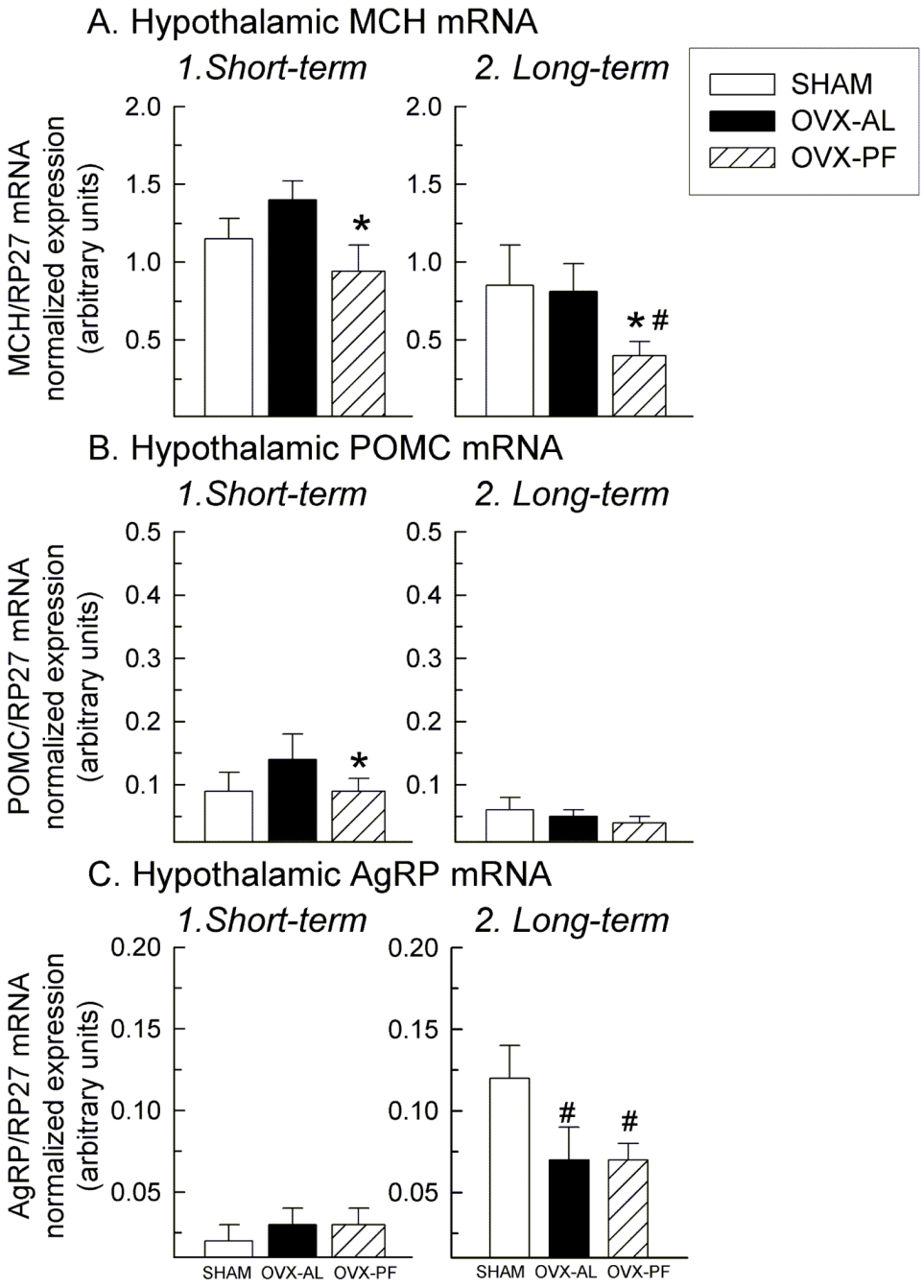

Fig. 6.

Hypothalamic MCH (*A; note: different scale*), POMC (*B*), and AgRP (*C*) mRNA, as measured by quantitative RT-PCR and normalized to expression of the housekeeping gene, RP27. Data represent mean ± SEM; (*) indicates $p < 0.05$ compared to OVX-AL, (#) indicates $p < 0.05$ compared to SHAM. *Abbreviations*: OVX, ovariectomy; AL, *ad libitum*; PF, pair-fed; MCH, melanin-concentrating hormone; POMC, pro-opiomelanocortin; AgRP, agouti-related peptide.

Table 1

Oligonucleotide primer sequences used for quantitative RT-PCR amplification.

Target	Primer sequence
AgRP	
Sense	5’- GCA GAC CGA GCA GAA GAT GT -3’
Antisense	5’ CTT GAA GAA GCG GCA GTA GC -3’
Prepro-MCH	
Sense	5’- TCG GTT GTT GCT CCT TCT CT -3
Antisense	5’- TTC CCT CTT TTC CTG TGT GG -3’
MCH-R1	
Sense	5’- TCA GCT TGG GCT ATG CTA ACA G -3
Antisense	5’- CAA CAC CAA GCG TTT TCG AA-3’
NPY	
Sense	5’- AGA GAT CCA GCC CTG AGA CA -3’
Antisense	5’- AAC GAC AAC AAG GGA AAT GG -3’
ObRb	
Sense	5’- CTG GGT TTG CGT ATG GAA GT -3’
Antisense	5’- CCA GTC TCT TGC TCC TCA CC -3’
ORX	
Sense	5’- ACC ACT GCA CCG AAG ATA CC -3’
Antisense	5’- AGT TCG TAG AGA CGG CAG GA -3’
POMC	
Sense	5’- GGT GTA CCC CAA TGT CG -3
Antisense	5’- CTT CTC GGA GGT CAT GAA GC -3’
RP27	
Sense	5’- TGG CGC TAA GAA AAG GAA GA -3’
Antisense	5’- ACC CAT GAA AAC TCC AGC AC -3’

AgRP: agouti-related peptide, **prepro-MCH**: prepro-melanin-concentrating hormone, **MCH-R1**: melanin-concentrating hormone receptor-1, **NPY**: neuropeptide-Y, **ObRb**: long isoform of the leptin receptor, **ORX**: orexin (a.k.a. hypocretin), **POMC**: proopiomelanocortin, and **RP27**: generic housekeeping gene.