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Article

Distinct Hypothalamic Neurons Mediate Estrogenic Effects on Energy Homeostasis and Reproduction

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SUMMARY

Estrogens regulate body weight and reproduction primarily through actions on estrogen receptor-α (ERα). However, ERα-expressing cells mediating these effects are not identified. We demonstrate that brain-specific deletion of ERα in female mice causes abdominal obesity stemming from both hyperphagia and hypometabolism. Hypometabolism and abdominal obesity, but not hyperphagia, are recapitulated in female mice lacking ERα in hypothalamic steroidogenic factor-1 (SF1) neurons. In contrast, deletion of ERα in hypothalamic pro-opiomelanocortin (POMC) neurons leads to hyperphagia, without directly influencing energy expenditure or fat distribution. Further, simultaneous deletion of ERα from both SF1 and POMC neurons causes hypometabolism, hyperphagia, and increased visceral adiposity. Additionally, female mice lacking ERα in SF1 neurons develop anovulation and infertility, while POMC-specific deletion of ERα inhibits negative feedback regulation of estrogens and impairs fertility in females. These results indicate that estrogens act on distinct hypothalamic ERα neurons to regulate different aspects of energy homeostasis and reproduction.

INTRODUCTION

Ovarian estrogens exert important antiobesity effects in women and female mammals. Lower levels of estrogens in postmenopausal women or in ovariectomized (OVX) animals are associated with obesity (Carr, 2003; Rogers et al., 2009). Estradiol-17β replacement in rodents prevents OVX-induced obesity by decreasing food intake and increasing energy expenditure (Gao et al., 2007). Hormone replacement therapy reverses the progression of obesity and metabolic dysfunctions in postmenopausal women (Wren, 2009). However, current hormone replacement therapy is often associated with increased prevalence of heart disease and breast cancer (Billeci et al., 2008). Because estrogens have both positive and negative effects on disease progression which are likely mediated by estrogen receptors (ERs) expressed in a variety of tissues, identification of the critical ERs and their sites of action is imperative in order to develop selective estrogen-based therapies which can selectively treat diseases associated with obesity.

Effects of estrogens on energy balance are primarily mediated by estrogen receptor-α (ERα), as women or female mice with mutations in the ERα gene display hyperadiposity (Heine et al., 2000; Okura et al., 2003), characteristically seen in postmenopausal women and OVX animals. However, the critical ERα sites that mediate estrogenic effects on energy homeostasis have not been identified. In the present study, we generated genetic mouse models with ERα selectively deleted in the central nervous system (CNS), in hypothalamic steroidogenic factor-1 (SF1) neurons, in pro-opiomelanocortin (POMC) neurons, or in both SF1 and POMC neurons, respectively. These models allowed us to identify ERα neuronal populations that regulate food intake, energy expenditure, fat distribution, and reproduction.

RESULTS

Loss of CNS ERα Impairs Multiple Aspects of Energy Homeostasis

Validation

To determine if CNS ERα is required for body weight control, we crossed mice carrying loxP-flanked ERα alleles ($ER\alpha^{lox/lox}$) to the Nestin-Cre transgenic mice. These crosses produced mice lacking ERα in most brain regions ($ER\alpha^{lox/lox}$/Nestin-Cre) and their control littermates ($ER\alpha^{lox/lox}$). Using immunohistochemistry, we demonstrated an almost complete absence of ERα in the hypothalamus (and other brain regions) in the $ER\alpha^{lox/lox}$/Nestin-Cre mice (see Figure S1 available online).

Increased Body Weight, Adiposity, and Visceral Fat Distribution

Compared to controls, both male and female $ER\alpha^{lox/lox}$/Nestin-Cre mice displayed significant increases in body weight

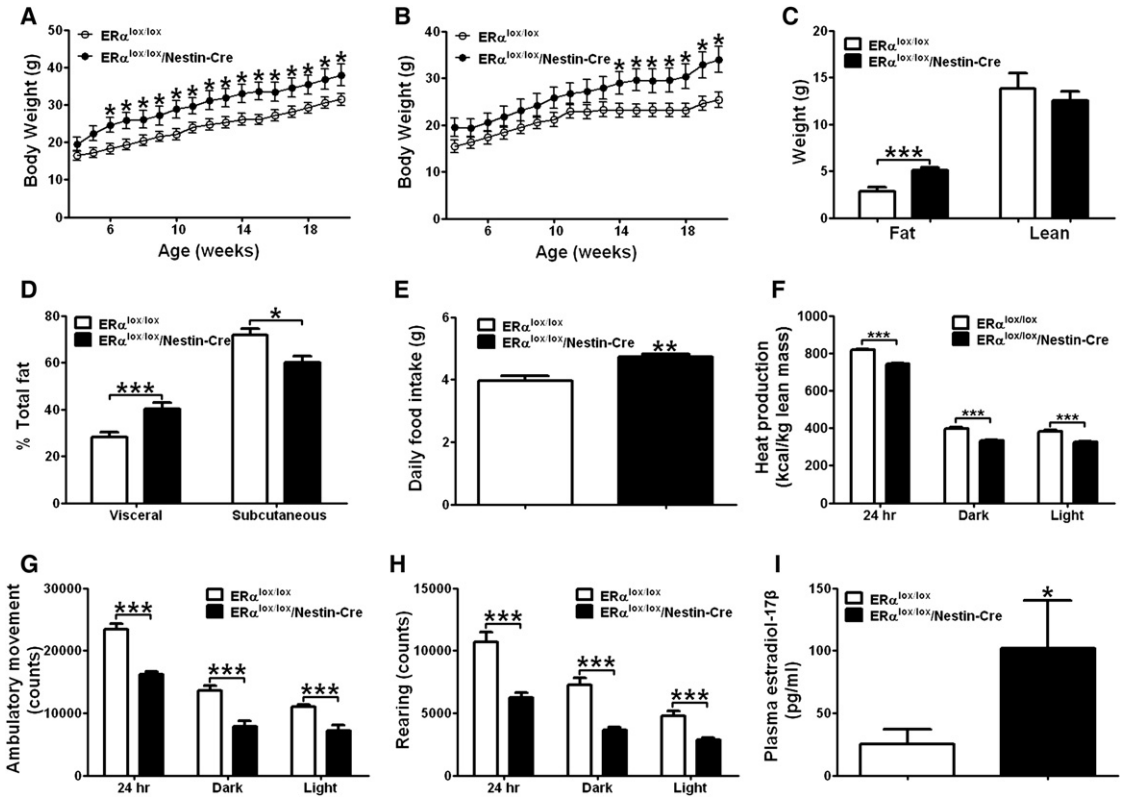

Figure 1. CNS ERα Regulates Energy Homeostasis

(A) Weekly body weight in male mice weaned on regular chow (n = 8/genotype).

(B) Weekly body weight in female mice weaned on regular chow (n = 8/genotype).

(C) Body composition in 15-week-old female mice fed with regular chow (n = 8/genotype).

(D) Relative fat distribution in the visceral and subcutaneous depots in 7-week-old female mice fed with regular chow (n = 8 or 10/genotype).

(E) Daily food intake in 7-week-old female mice fed with regular chow (n = 8 or10/genotype).

(F–H) Chow-fed female mice (n = 8 or 10/genotype) were acclimated to the TSE metabolic chambers. Average heat production (F), ambulatory movements (G), and rearing activities (H) during 24 hr, 12 hr dark cycle and 12 hr light cycle. Note that mice used in (F)–(H) were 12-week-old littermates and had comparable body weight ($ER\alpha^{lox/lox}$, 22.8 ± 1.5 versus $ER\alpha^{lox/lox}$/Nestin-Cre, 27.2 ± 2.5, $p > 0.05$) and lean mass ($ER\alpha^{lox/lox}$, 13.80 ± 1.72 versus $ER\alpha^{lox/lox}$/Nestin-Cre, 12.50 ± 0.98, $p > 0.05$), but different fat mass ($ER\alpha^{lox/lox}$, 2.80 ± 0.45 versus $ER\alpha^{lox/lox}$/Nestin-Cre, 5.10 ± 0.27, $p < 0.001$).

(I) Plasma estradiol-17β at diestrus in 7-week-old female mice fed with regular chow (n = 8 or 10/genotype). Note that mice used in (D), (E), and (I) had different total fat mass ($ER\alpha^{lox/lox}$, 3.53 ± 0.48 versus $ER\alpha^{lox/lox}$/Nestin-Cre, 6.01 ± 1.67, $p < 0.05$) but comparable lean mass ($ER\alpha^{lox/lox}$, 13.51 ± 0.21 versus $ER\alpha^{lox/lox}$/Nestin-Cre, 14.10 ± 0.76 ($p > 0.05$). Data are presented as mean ±SEM, and *$p < 0.05$ and ***$p < 0.001$ between $ER\alpha^{lox/lox}$/Nestin-Cre mice and $ER\alpha^{lox/lox}$ mice.

(Figures 1A and 1B). Further characterizations in female mice revealed that increases in body weight were mainly reflected by increased body fat mass (Figure 1C). We further demonstrated that $ER\alpha^{lox/lox}$/Nestin-Cre female mice had significantly higher visceral fat distribution (percent of the whole-body fat) but lower subcutaneous fat distribution (Figure 1D). These data indicate that CNS ERα is required to regulate body weight, adiposity, and fat distribution.

Hyperphagia, and Decreased Energy Expenditure and Physical Activity

$ER\alpha^{lox/lox}$/Nestin-Cre mice displayed hyperphagia (Figure 1E) and decreased heat production (Figure 1F). The lower energy expenditure may be partly caused by decreased physical activity, as the ambulatory movements and rearing activities in $ER\alpha^{lox/lox}$/Nestin-Cre mice were significantly reduced (Figures 1G and 1H). These results demonstrate that CNS ERα is required to mediate estrogenic effects on feeding, energy expenditure, and physical activity.

Elevated Plasma Estradiol-17β

Notably, we found that circulating estradiol-17β was significantly elevated in $ER\alpha^{lox/lox}$/Nestin-Cre mice (Figure 1I). Our observations that mice lacking ERα only in the CNS develop obesity despite the elevated levels of estradiol-17β in the circulation suggest that, compared to ERα expressed in the peripheral tissues, CNS ERα appears to play more predominant roles in the regulation of energy balance.

Loss of ERα in VMH SF1 Neurons Affects Energy Expenditure and Fat Distribution

Validation

The ventromedial hypothalamic nucleus (VMH) is important for regulation of body weight (King, 2006). We hypothesized that ERα in the VMH is required to mediate the estrogenic effects on energy balance. Because SF1, a transcription factor, is expressed exclusively in the VMH within the brain (Ikeda et al., 1995), we used a SF1-Cre transgenic mice (line 7) (Dhillon

et al., 2006) to generate mice lacking ERα in the VMH (ERα$^{lox/lox}$/SF1-Cre). In control (SF1-Cre/rosa26GFP) mice, 48.3% ± 5.7% of ERα neurons in the VMH coexpress SF1, and 12.0% ± 1.9% of SF1 neurons express ERα. In ERα$^{lox/lox}$/SF1-Cre/rosa26GFP mice, the majority of ERα was selectively deleted from the VMH SF1 neurons (Figure S2). Importantly, we found that the numbers of SF1neurons in ERα$^{lox/lox}$/SF1-Cre/rosa26GFP mice and in control mice were comparable (Figure S2), suggesting that deletion of ERα did not cause loss of the SF1 neuronal population in the VMH.

Increased Body Weight, Adiposity, and Visceral Fat Distribution

When fed on regular chow, male ERα$^{lox/lox}$/SF1-Cre and control (ERα$^{lox/lox}$) littermates had comparable body weight and body composition (Figures 2A and 2B). The chow-fed ERα$^{lox/lox}$/SF1-Cre females had modest but significant increases in body weight (Figure 2C) and had increases in both fat mass and lean mass (Figure 2D). When fed with high-fat diet (HFD), male ERα$^{lox/lox}$/SF1-Cre and control mice showed comparable body weight (Figure 2E) and fat/lean mass (Figure 2F). On the other hand, HFD-fed ERα$^{lox/lox}$/SF1-Cre females had significant increases in body weight compared to control mice (Figure 2G), which were reflected by increases in both fat mass and lean mass (Figure 2H). Collectively, these results indicate that ERα expressed by SF1 cells is required to maintain body weight homeostasis in females, but not in males.

ERα$^{lox/lox}$/SF1-Cre females displayed significantly higher visceral fat distribution but lower subcutaneous fat distribution than controls (Figure 2I). Interestingly, 30% of adult ERα$^{lox/lox}$/SF1-Cre females had a massive accumulation of gonadal adipose tissue as demonstrated by appearance (Figure 2J) and weight (Figure 2K). Although it is unclear why the gonadal fat pads in subsets of mutant mice form this peculiar shape, this phenomenon is consistent with the abdominal obese phenotype. Given that increased visceral fat is commonly associated with impaired glucose homeostasis (Lafontan and Girard, 2008), we performed glucose tolerance tests and found that ERα$^{lox/lox}$/SF1-Cre females were glucose intolerant (Figure 2L). Collectively, these results indicate that ERα in SF1 neurons is required for the regulation of fat distribution. Loss of ERα in SF1 neurons leads to abdominal obesity, which subsequently causes glucose dysregulation.

We further characterized the white adipose tissue (WAT) in these mutant females. We found that the average adipocyte size of gonadal WAT was larger in ERα$^{lox/lox}$/SF1-Cre mice (Figures 2M and 2N). Importantly, compared to controls, ERα$^{lox/lox}$/SF1-Cre females stored more energy in the gonadal WAT, demonstrated by significantly increased triglyceride content (normalized by fat weight) (Figure 2P). Consistently, mRNA levels of genes promoting lipogenesis and triglyceride storage, including stearoyl-CoA desaturase-1 (SCD1), lipoprotein lipase (LPL), and acetyl-CoA carboxylase α (ACCα), were significantly elevated in gonadal WAT of ERα$^{lox/lox}$/SF1-Cre females (Figure 2Q). These findings indicate that ERα in SF1 neurons is required for the regulation of energy partitioning in the gonadal WAT. On the other hand, the inguinal WAT of ERα$^{lox/lox}$/SF1-Cre mice and controls had comparable adipocyte size (Figures 2M and 2O), triglyceride content (Figure 2P), and gene expression profile (Figure 2Q). No significant difference was observed in the expression of fatty acid synthase (FAS), ERα, and ERβ in all the fat pads (Figure 2Q).

Decreased Energy Expenditure

The increased body weight and adiposity in ERα$^{lox/lox}$/SF1-Cre females was not due to differences in energy intake, because young or adult female ERα$^{lox/lox}$/SF1-Cre and control mice consumed comparable levels of calories (Figure 3A). In contrast, ERα$^{lox/lox}$/SF1-Cre females were hypometabolic, as demonstrated by significant decreases in heat production (Figure 3B). Components of total energy expenditure include energy required for physical activities, basal metabolism, and diet-induced thermogenesis (Castaneda et al., 2005). In particular, ERα$^{lox/lox}$/SF1-Cre females showed normal ambulatory movements and rearing activities (Figures 3C and 3D), indicating that ERα in SF1 neurons is not required to regulate physical activity. We further assessed the basal metabolic rate by measuring the minimal heat production during the light cycle (Kaiyala et al., 2010) and found that ERα$^{lox/lox}$/SF1-Cre females had significant reductions in basal metabolic rate (Figure 3E). To assess diet-induced thermogenesis, we monitored energy expenditure in response to a fasting-refeeding paradigm. The increased heat production in ERα$^{lox/lox}$/SF1-Cre mice was significantly reduced (Figures 3F and 3G). Thus, our results suggest that ERα expressed by VMH SF1 neurons is required to regulate basal metabolic rate and to mediate appropriate thermogenic responses to feeding.

Impaired BAT Thermogenesis

Brown adipose tissue (BAT) plays crucial roles in mediating thermogenesis (Dulloo, 2002). Although the weight of BAT was not different between genotypes (ERα$^{lox/lox}$, 86.67 ± 7.68 mg versus ERα$^{lox/lox}$/SF1-Cre, 97.86 ± 7.16 mg, $p > 0.05$, $n = 12$ or 15/genotype), histological analyses revealed a large amount of lipid deposition in the ERα$^{lox/lox}$/SF1-Cre BAT (Figure 3H). Consistently, UCP1 mRNA levels in the ERα$^{lox/lox}$/SF1-Cre BAT were significantly reduced (Figure 3I). In addition, mRNA levels of peroxisome proliferator-activated receptor γ (PPARγ), PPARγ coactivator-1α (PGC-1α), and β3 adrenergic receptor, factors known to stimulate UCP1 expression (Seale et al., 2007), were significantly lower in ERα$^{lox/lox}$/SF1-Cre BAT (Figure 3I). The levels of PRDM16 and ERα in BAT were not altered (Figure 3I). Taken together, these findings suggest that reduced ERα signals in VMH SF1 neurons led to a reduction in thermogenic functions of BAT by suppressing UCP1 expression.

Decreased Sympathetic Outflow

ERα$^{lox/lox}$/SF1-Cre females had significantly lower plasma norepinephrine levels than controls; plasma epinephrine levels were not significantly different (Figure 3J). These findings suggest that ERα in VMH SF1 neurons is required to maintain normal central sympathetic outflow to the peripheral tissues. The decreased sympathetic tone in ERα$^{lox/lox}$/SF1-Cre mice could contribute to decreased BAT thermogenesis and the increased lipid accumulation in gonadal WAT.

Decreased Expression of Leptin Receptors

Messenger RNA of leptin receptors was significantly reduced in the VMH of ERα$^{lox/lox}$/SF1-Cre females (Figure 3K). These findings support the possibility that ERα signals in SF1 neurons are required to maintain normal leptin sensitivity by regulating transcription of leptin receptors. No significant changes were found in the mRNA levels of SF1, ERβ, thyroid hormone

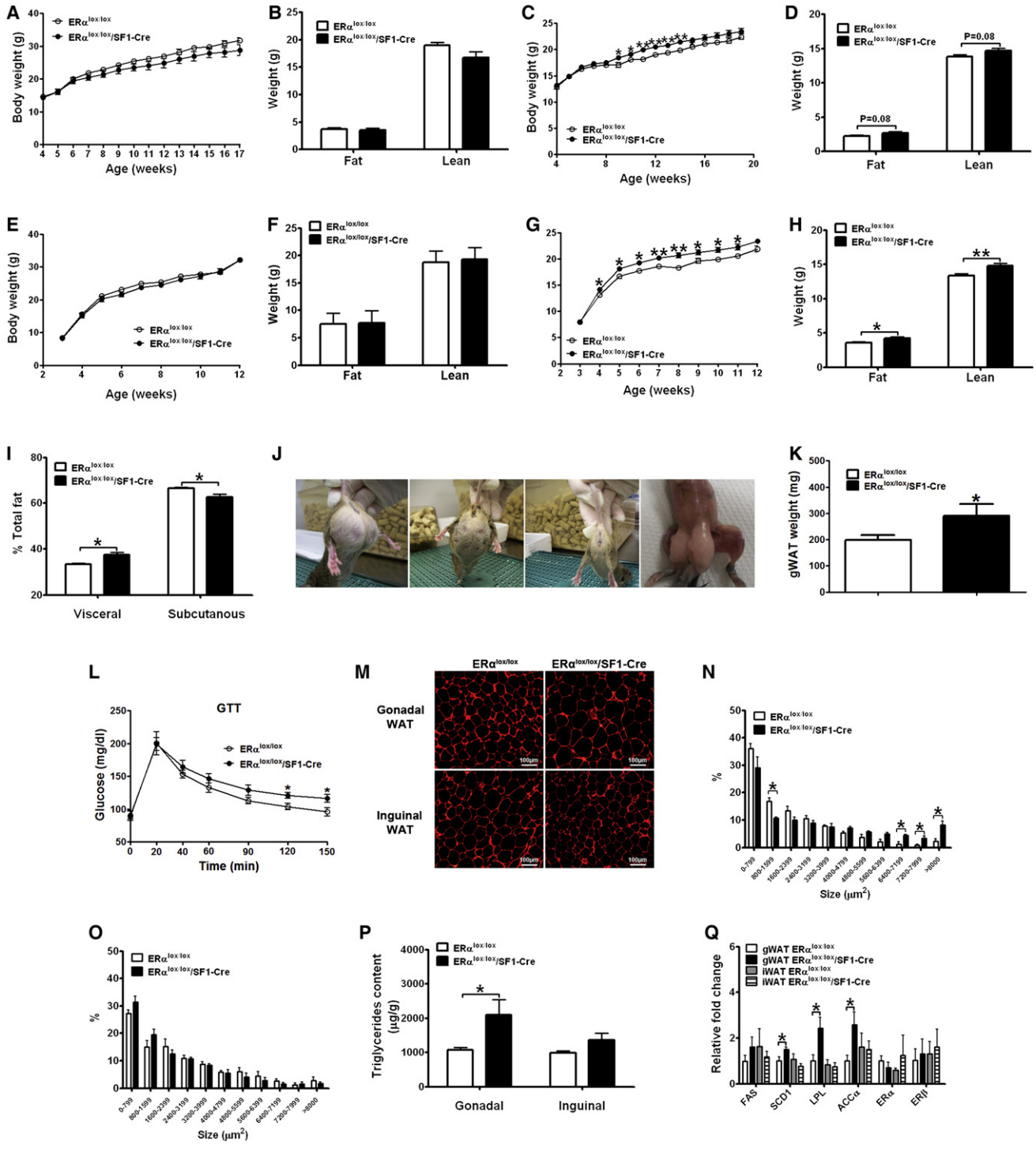

Figure 2. ERα Expressed by SF1 Neurons Regulates Body Weight and Fat Distribution

(A) Weekly body weight in male mice weaned on regular chow (n = 6 or 12/genotype).
(B) Body composition in 11-week-old male mice fed with regular chow (n = 6 or 12/genotype).
(C) Weekly body weight in female mice weaned on regular chow (n = 22 or 25/genotype).
(D) Body composition in 11-week- old female mice fed with regular chow (n = 22 or 25/genotype).
(E) Weekly body weight in male mice weaned on HFD (n = 11 or 21/genotype).
(F) Body composition in 12-week-old male mice fed with HFD (n = 11 or 21/genotype).
(G) Weekly body weight in female mice weaned on HFD (n = 10 or 15/genotype).
(H) Body composition in 8-week-old female mice fed with HFD (n = 10 or 15/genotype).

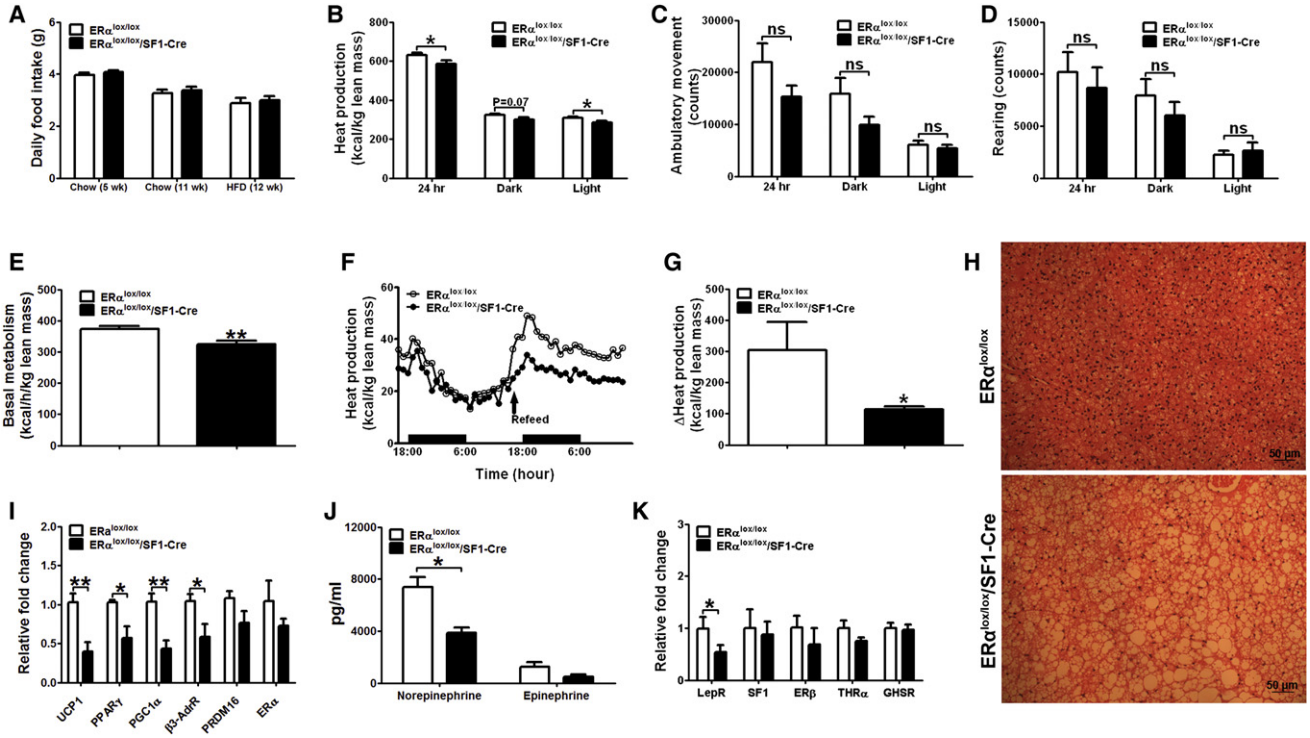

Figure 3. ERα Expressed by SF1 Neurons Regulates Energy Expenditure

(A) Daily food intake in 5-week-old female mice fed with regular chow (n = 7 or 23/genotype), 11-week-old female mice fed with regular chow (n = 9/genotype), and 12-week-old female mice fed with HFD (n = 12/genotype).

(B–E) Chow-fed female mice were acclimated to the TSE metabolic chambers (n = 11 or 12/genotype). Shown are average heat production (B), ambulatory movements (C), and rearing activities (D) during 24 hr, 12 hr dark cycle and 12 hr light cycle, and basal metabolism (E). Note that mice used in (B)–(E) were 20-week-old littermates and had comparable body weight ($ER\alpha^{lox/lox}$, 20.26 ± 0.47 versus $ER\alpha^{lox/lox}$/SF1-Cre: 21.31 ± 0.53, $p > 0.05$), fat mass ($ER\alpha^{lox/lox}$, 3.37 ± 0.26 versus $ER\alpha^{lox/lox}$/SF1-Cre, 4.10 ± 0.45, $p > 0.05$), and lean mass ($ER\alpha^{lox/lox}$, 15.45 ± 0.45 versus $ER\alpha^{lox/lox}$/SF1-Cre, 15.97 ± 0.34, $p > 0.05$).

(F and G) HFD-fed female mice were acclimated to the TSE metabolic chambers. HFD was removed from the TSE metabolic chambers for 24 hr, followed by refeeding for 24 hr. Temporal responses in heat production (F) of female mice during fasting and HFD refeeding were monitored. (G) Increases in heat production after HFD refeeding were calculated by subtracting heat production during 24 hr fasting from heat production during 24 hr refeeding. Note that mice used in (F) and (G) were 12-week-old littermates and had comparable body weight ($ER\alpha^{lox/lox}$, 20.75 ± 0.39 versus $ER\alpha^{lox/lox}$/SF1-Cre, 21.05 ± 0.65, $p > 0.05$) but different fat mass ($ER\alpha^{lox/lox}$, 3.55 ± 0.19 versus $ER\alpha^{lox/lox}$/SF1-Cre, 4.23 ± 0.22, $p < 0.05$) and lean mass ($ER\alpha^{lox/lox}$, 13.55 ± 0.32 versus $ER\alpha^{lox/lox}$/SF1-Cre, 14.77 ± 0.34, $p < 0.05$).

(H) Representative photomicrographs of H&E staining of BAT from 16-week-old HFD-fed females.

(I) Messenger RNA levels in BAT from 16-week-old HFD-fed females (n = 6/genotype).

(J) Plasma norepinephrine and epinephrine in chow-fed females (n = 6/genotype).

(K) Messenger RNA levels in microdissected VMH from 16-week-old HFD-fed females (n = 6/genotype). Data are presented as mean ±SEM, and *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ between $ER\alpha^{lox/lox}$/SF1-Cre mice and $ER\alpha^{lox/lox}$ mice.

receptor-α (THRα), and growth hormone secretagogue receptor (GHSR, ghrelin receptors) in the VMH of $ER\alpha^{lox/lox}$/SF1-Cre females (Figure 3K).

Responses in the Pituitary, Adrenal Gland, and Gonads

In addition to the VMH in the brain, SF1 cells are also found in the pituitary, adrenal gland, and gonads (Zhao et al., 2001). Therefore, ERα may be deleted in these endocrine organs, which may potentially confound the metabolic phenotypes. We found that ERα mRNA levels were comparable in these organs from the two genotypes (Figure S3A). These results suggest that ERα may not be expressed in SF1 cells in these peripheral organs in wild-type mice, and therefore SF1-Cre-induced recombination

(I) Relative fat distribution in the visceral and subcutaneous depots in 8-week-old female mice fed with HFD (n = 5/genotype).

(J) Photographs of two $ER\alpha^{lox/lox}$/SF1-Cre female mice (the first and second from the left) and one $ER\alpha^{lox/lox}$ female (the third from the left); the photograph of the carcass of one $ER\alpha^{lox/lox}$/SF1-Cre female (the fourth from the left; note that the abdominal wall of the mouse was intact).

(K) Weight of gonadal WAT in 6-week-old female mice fed with regular chow (n = 6 or 9/genotype).

(L) Glucose tolerance test in 10-week-old chow-fed female mice (i.p. 1 g/kg glucose, n = 8 or 11/genotype).

(M) Representative photomicrographs of H&E staining of gonadal WAT and inguinal WAT from 16-week-old HFD-fed females.

(N and O) Cell size in gonadal WAT (N) and inguinal WAT (O) from 16-week-old HFD-fed females (n = 3 or 4/genotype).

(P) Triglyceride content in gonadal WAT and inguinal WAT from 16-week-old HFD-fed $ER\alpha^{lox/lox}$ and $ER\alpha^{lox/lox}$/SF1-Cre females (n = 6/genotype).

(Q) Messenger RNA levels in WAT from 16-week-old HFD-fed females (n = 6/genotype). Data are presented as mean ±SEM, and *$p < 0.05$ and **$p < 0.01$ between $ER\alpha^{lox/lox}$/SF1-Cre mice and $ER\alpha^{lox/lox}$ mice.

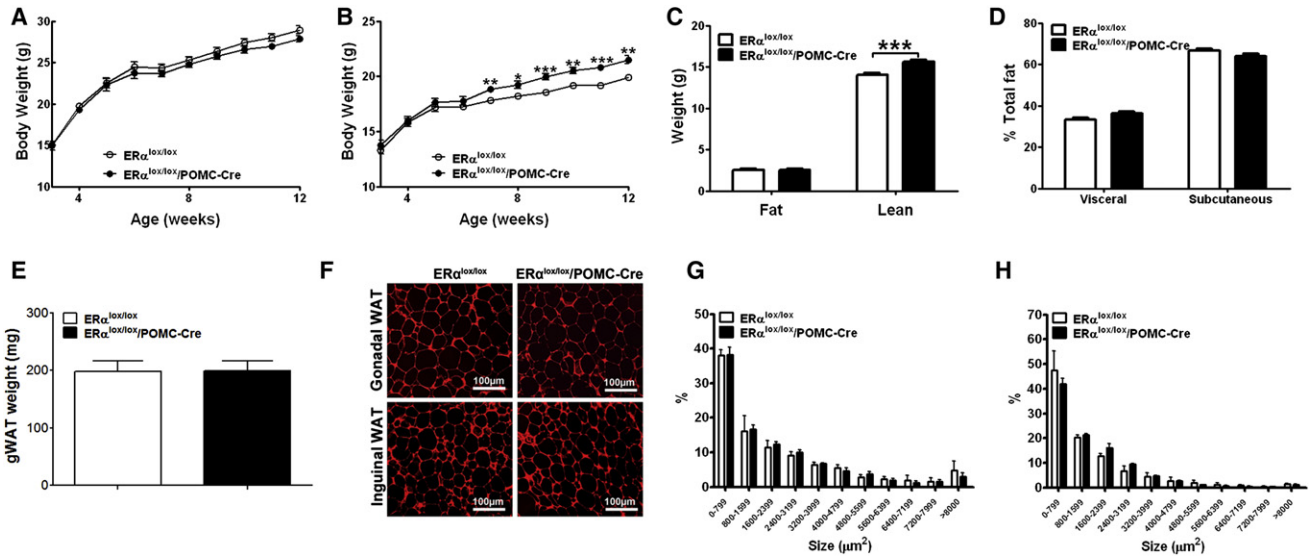

Figure 4. Deletion of ERα in POMC Neurons Leads to Increased Body Weight and Lean Mass

(A) Weekly body weight in male mice weaned on regular chow (n = 33 or 34/genotype).

(B) Weekly body weight in female mice weaned on regular chow (n = 25 or 32/genotype).

(C) Body composition in 11-week-old female mice fed with regular chow (n = 25 or 32/genotype).

(D) Relative fat distribution in the visceral and subcutaneous depots in 18-week-old female mice fed with regular chow (n = 5/genotype).

(E) Weight of gonadal WAT in 6-week-old female mice fed with regular chow (n = 8 or 9/genotype).

(F) Representative photomicrographs of H&E staining of gonadal WAT and inguinal WAT from 5-month-old chow-fed females.

(G and H) Cell size in gonadal WAT (G) and inguinal WAT (h) from 5-month-old chow-fed females (n = 3/genotype). Data are presented as mean ±SEM, and $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ between $ER\alpha^{lox/lox}$/POMC-Cre mice and $ER\alpha^{lox/lox}$ mice.

did not affect ERα expression. Alternatively, the loss of ERα from these peripheral SF1 cells, if any, may be compensated by elevated ERα expressed by non-SF1 cells in the same organs. We further examined the impact of the possible ERα deletion on the functions of these organs. We found that there were no significant changes in the plasma estradiol-17β, corticosterone, T3/T4, progesterone, FSH, or LH (Table S1). No major abnormalities were observed in the morphology of the pituitary and adrenal gland (Figure S3B). However, in $ER\alpha^{lox/lox}$/SF1-Cre mice, their ovaries showed an increased number of antral follicles and lack of corpus luteum (Figure S3B), consistent with our findings that $ER\alpha^{lox/lox}$/SF1-Cre females were infertile (data not shown). Nevertheless, because the hormones secreted from the pituitary, adrenal gland, and ovary were not significantly altered, it is unlikely that the metabolic phenotypes outlined above are due to the possible deletion of ERα in these peripheral organs, although this possibility cannot be fully excluded.

Loss of ERα in POMC Neurons Directly Affects Feeding and Negative Feedback

Validation

POMC neurons secrete α-melanocyte-stimulating hormone (α-MSH) to reduce food intake and increase energy expenditure (Morton et al., 2006). Estrogens activate POMC neurons (Gao et al., 2007; Malyala et al., 2008). However, the physiological significance of ERα expressed by POMC neurons has not been directly tested. We crossed $ER\alpha^{lox/lox}$ mice to the POMC-Cre transgenic mice to generate mice lacking ERα in POMC neurons ($ER\alpha^{lox/lox}$/POMC-Cre). In control (POMC-Cre/rosa26GFP) mice, 21.5% ± 2.5% of POMC neurons in the ARH and 20.9% ± 4.8% of POMC neurons in the NTS coexpressed ERα (Figure S4). In $ER\alpha^{lox/lox}$/POMC-Cre/rosa26GFP mice, only 1.9% ± 0.4% of POMC neurons in the ARH and none in the NTS coexpressed ERα, confirming that the majority of ERα was selectively deleted from POMC neurons (Figure S4). Importantly, deletion of ERα from POMC neurons did not cause loss of POMC neurons (Figure S4).

Increased Body Weight

Male $ER\alpha^{lox/lox}$/POMC-Cre and control ($ER\alpha^{lox/lox}$) littermates showed comparable body weight (Figure 4A). On the other hand, $ER\alpha^{lox/lox}$/POMC-Cre females had significant increases in body weight (Figure 4B), mainly reflected by increases in lean mass (Figure 4C). No significant differences were observed in the visceral/subcutaneous fat distribution (Figure 4D), the weight of gonadal fat pads (Figure 4E), and the adipocyte size in gonadal and inguinal WAT (Figures 4F–4H) between the $ER\alpha^{lox/lox}$/POMC-Cre females and controls.

Hyperphagia and Blunted Anorexigenic Responses to Leptin

$ER\alpha^{lox/lox}$/POMC-Cre females displayed a chronic hyperphagia. This is demonstrated by significant increases in food intake in chow-fed $ER\alpha^{lox/lox}$/POMC-Cre females at 4 and 6 months of age (Figure 5A). When normalized by lean mass, food intake was significantly increased at 6 months (Figure 5B). We further examined the effects of anorexigenic hormones in these females. While leptin (5 mg/kg, i.p.) significantly reduced food intake in controls, these anorexigenic effects were blunted in $ER\alpha^{lox/lox}$/POMC-Cre females (Figure 5C). In contrast, anorexia

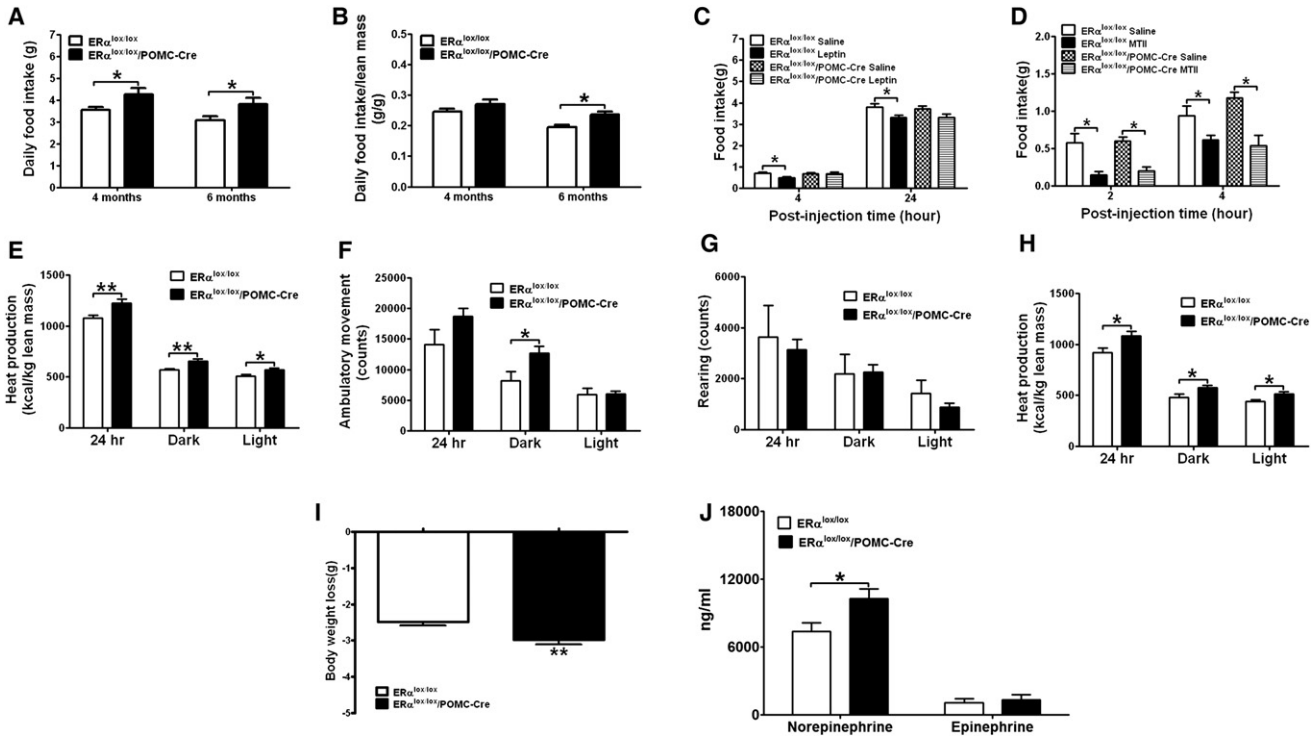

Figure 5. Deletion of ERα in POMC Neurons Leads to Hyperphagic and Hypermetabolic Phenotypes

(A) Daily food intake in 4-month-old and 6-month-old female mice fed with regular chow (n = 12 or 13/genotype).

(B) The same food intake data in (A) presented as daily food intake normalized by lean mass.

(C) Effects of i.p. injections of leptin (5 mg/kg) on food intake in 12-week-old chow-fed female mice (n = 8/group).

(D) Effects of i.p. injections of MTII (1 mg/kg) on food intake in 16-week-old chow-fed female mice (n = 8/group).

(E–I) HFD-fed female mice (n = 10 or 13/genotype) were acclimated to the TSE metabolic chambers. Average heat production (E), ambulatory movements (F), and rearing activities (G) during 24 hr, 12 hr dark cycle and 12 hr light cycle, were measured at fed condition. (H and I) HFD was removed from the TSE metabolic chambers for 24 hr, and average heat production (H) during 24 hr, 12 hr dark cycle and 12 hr light cycle, was measured at fasted condition. (I) Body weight loss induced by 24 hr fasting was measured. Note that mice used in (E)–(I) were 20-week-old littermates and had comparable body weight ($ER\alpha^{lox/lox}$, 25.14 ± 1.16 versus $ER\alpha^{lox/lox}$/POMC-Cre, 25.12 ± 0.95, $p > 0.05$), fat mass ($ER\alpha^{lox/lox}$, 8.84 ± 0.13 versus $ER\alpha^{lox/lox}$/POMC-Cre, 8.80 ± 0.11, $p > 0.05$), and lean mass ($ER\alpha^{lox/lox}$, 14.28 ± 0.27 versus $ER\alpha^{lox/lox}$/POMC-Cre, 14.72 ± 0.22, $p > 0.05$).

(J) Plasma norepinephrine and epinephrine in chow-fed females (n = 6/genotype). Data are presented as mean ±SEM, and *$p < 0.05$ and **$p < 0.01$ between $ER\alpha^{lox/lox}$/POMC-Cre mice and $ER\alpha^{lox/lox}$ mice.

induced by MTII, a melanocortin agonist, was indistinguishable in $ER\alpha^{lox/lox}$/POMC-Cre and controls (Figure 5D). Collectively, these findings demonstrated that ERα in POMC neurons is required to maintain normal feeding behavior, at least partly through interacting with the anorexigenic leptin signals.

Increased Energy Expenditure, Sympathetic Outflow, and Plasma Estradiol-17β

Unexpectedly, ad libitum $ER\alpha^{lox/lox}$/POMC-Cre females showed increased heat production (Figure 5E). In addition, the ambulatory movements during the dark cycle were significantly elevated in the $ER\alpha^{lox/lox}$/POMC-Cre females (Figure 5F), which may at least partly contribute to the increased energy expenditure. The rearing activities were comparable between the two genotypes (Figure 5G). To further confirm the unexpected hypermetabolic phenotypes, we examined the energy expenditure during fasting. Consistent with results from ad libitum mice, $ER\alpha^{lox/lox}$/POMC-Cre mice showed increased heat production during 24 hr fasting (Figure 5H), which led to a significantly greater weight loss (Figure 5I). Interestingly, $ER\alpha^{lox/lox}$/POMC-Cre females had significantly higher plasma norepinephrine than control mice, while plasma epinephrine levels were comparable (Figure 5J). The elevated sympathetic tone may be the underlying mechanisms for elevated metabolism seen in these $ER\alpha^{lox/lox}$/POMC-Cre mice.

Although we cannot fully exclude the possibility that the increases in sympathetic outflow and energy expenditure in $ER\alpha^{lox/lox}$/POMC-Cre females are directly due to ERα deletion from POMC neurons, it is more likely that these phenotypes may result from compensatory actions of ERα in other CNS sites. Supporting this notion, we found that $ER\alpha^{lox/lox}$/POMC-Cre females have significantly higher plasma estradiol-17β levels (Figure 6A). Presumably, the elevated estradiol-17β would act on other CNS ERα sites (including SF1 neurons) to increase sympathetic outflow, energy expenditure, and physical activity.

Impaired Negative Feedback and Fertility

Plasma progesterone in $ER\alpha^{lox/lox}$/POMC-Cre females trended to increase but failed to reach statistical significance (Figure 6B). Although the basal FSH and LH levels in gonad intact females were not significantly altered (Table S2), the estrogen-induced

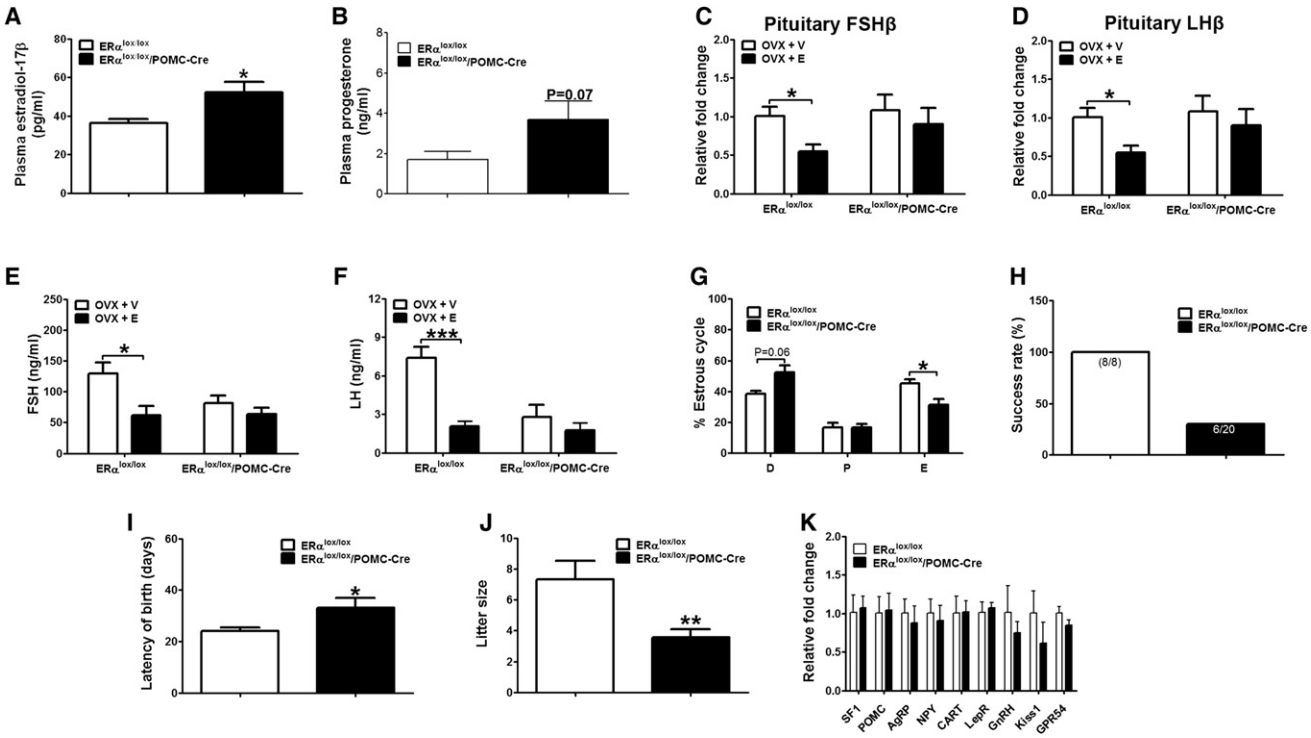

Figure 6. Deletion of ERα in POMC Neurons Leads to Fertility Phenotypes

(A and B) Plasma estradiol-17β (A) and progesterone (B) at diestrus in 6-month-old female mice fed with HFD (n = 6 or 8/genotype).

(C and D) Relative mRNA levels of FSHβ (C) or LHβ (D) in the pituitary measured 4 weeks after receiving ovriectomy plus estradiol-17β replacement (0.5 μg/day/mouse, OVX+E) or plus vehicle (OVX+V) (n = 6/group).

(E and F) Plasma FSH (E) or LH (F) measured in mice described in (C) and (D).

(G) Length of diestrus, proestrus, and estrus relative to the entire etrus cycles (n = 8 or 17/genotype).

(H) Percentage of mice that successfully delivered pups (n = 8 or 20/genotype).

(I) Averaged time period between mating day and birth day of pups (n = 8 or 6/genotype).

(J) Averaged litter size (n = 8 or 6/genotype). Note that only mice that successfully delivered pups in (H) were included in the analyses in (I) and (J).

(K) Relative mRNA levels in the hypothalamus from 16-week-old chow-fed females (n = 6/genotype). Data are presented as mean ±SEM, and *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ between ERα$^{lox/lox}$/POMC-Cre mice and ERα$^{lox/lox}$ mice.

suppressions of FSH and LH were blunted in ERα$^{lox/lox}$/POMC-Cre females. Specifically, estradiol-17β replacement in OVX control females significantly suppressed expression of FSH and LH subunits in the pituitary, whereas these negative feedback effects were blunted in ERα$^{lox/lox}$/POMC-Cre females (Figures 6C and 6D). Similar patterns were also observed in plasma FSH and LH (Figures 6E and 6F). These results suggest that ERα in POMC neurons is required to mediate the negative feedback regulation. Of note, the plasma FSH/LH levels after OVX appeared to be lower in ERα$^{lox/lox}$/POMC-Cre compared to control mice (Figures 6E and 6F), while the pituitary mRNA levels of FSH/LH subunits in the same mice were comparable (Figures 6C and 6D). This discrepancy suggests that the secretion of FSH/LH or the stability/degradation of plasma FSH/LH may be differently regulated in the OVX ERα$^{lox/lox}$/POMC-Cre mice.

In addition, we found that the gonad-intact ERα$^{lox/lox}$/POMC-Cre females had abnormal estrous cycles. The length of the estrous phase was significantly reduced, while the diestrus phase trended to be elongated in ERα$^{lox/lox}$/POMC-Cre mice (Figure 6G). No differences were observed in the length of the proestrus phase (Figure 6G). Further, female ERα$^{lox/lox}$/POMC-Cre mice had impaired reproductive capacity. In particular, only 30% of ERα$^{lox/lox}$/POMC-Cre females (6 out of 20) were able to conceive and deliver, while 100% of the age-matched control females gave birth (Figure 6H). In addition, it took significantly longer for the six ERα$^{lox/lox}$/POMC-Cre dams to conceive than the controls (Figure 6I). The average litter size from these dams was significantly reduced (Figure 6J). No major abnormalities were observed in the morphology of ovaries from ERα$^{lox/lox}$/POMC-Cre females (Figure S5C). Collectively, these findings indicate that ERα in POMC neurons is required to maintain normal estrous cyclicity and female fertility.

Expression of Hypothalamic Genes

In an attempt to further characterize the effects of estrogen/ERα on POMC neurons, we examined the gene expression profile in the hypothalamus of ERα$^{lox/lox}$/POMC-Cre females. However, there was no significant difference in the expression of POMC, AgRP, NPY, CART, leptin receptors, SF1, GnRH, Kiss1, and GPR54 between ERα$^{lox/lox}$/POMC-Cre females and controls (Figure 6K).

Responses in Pituitary Functions

In addition to the POMC neurons in the brain, POMC (ACTH) cells also exist in the pituitary. We found that ERα mRNA was significantly reduced in the ERα$^{lox/lox}$/POMC-Cre pituitary (Figure S5A),

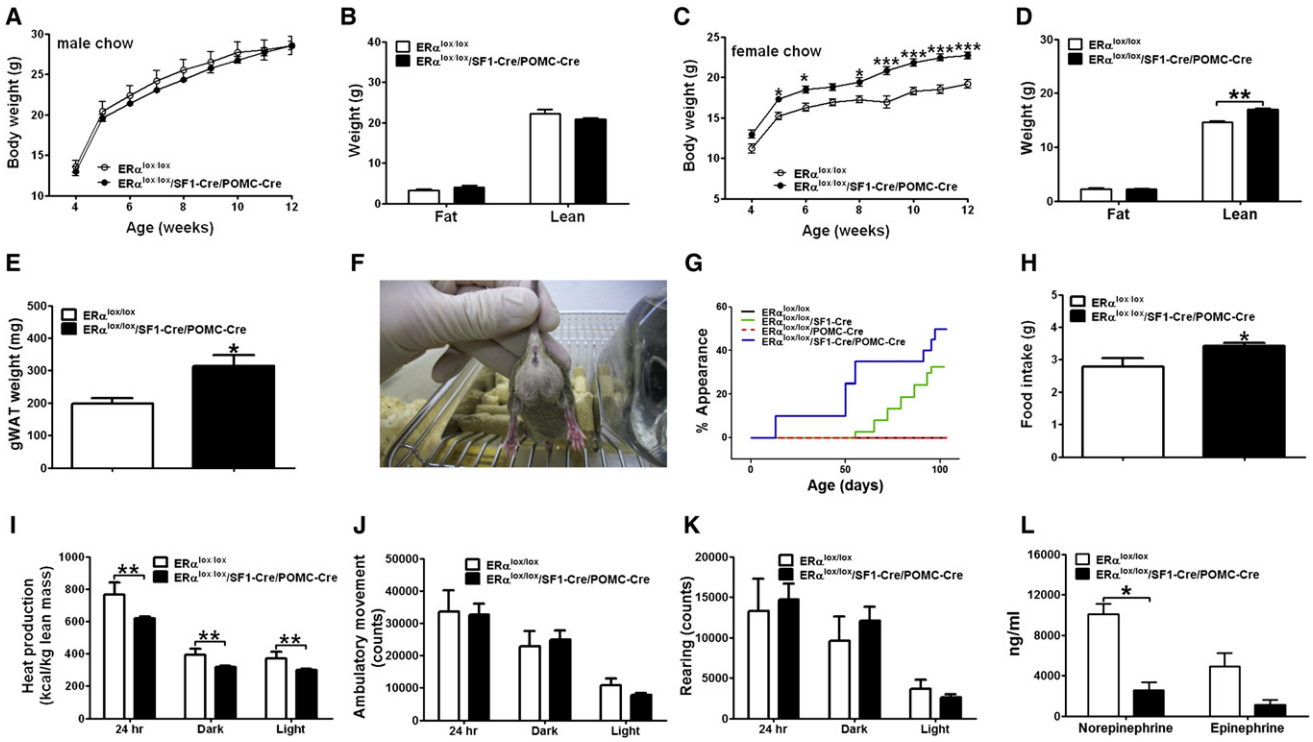

Figure 7. Deletion of ERα in Both SF1 and POMC Neurons Produces Hyperphagia and Decreased Energy Expenditure

(A) Weekly body weight in male mice weaned on regular chow (n = 6 or 15/genotype).

(B) Body composition in 12-week-old male mice fed with regular chow (n = 6 or 15/genotype).

(C) Weekly body weight in female mice weaned on regular chow (n = 11 or 39/genotype).

(D) Body composition in 12-week-old female mice fed with regular chow (n = 11 or 39/genotype).

(E) Weight of gonadal WAT in 6-week-old female mice fed with regular chow (n = 7 or 9/genotype).

(F) Photograph of an ERα$^{lox/lox}$/SF1-Cre/POMC-Cre female mouse at the age of 6 weeks.

(G) Appearance rate of abdominal obesity (defined by obvious abnormal expansion of the lower abdominal obesity).

(H) Daily food intake was measured in 6-week-old female mice fed with regular chow (n = 6 or 17/genotype).

(I–K) Chow-fed female mice (n = 6 or 17/genotype) were acclimated to the TSE metabolic chambers, and average heat production (I), ambulatory movement (J), and rearing activity (K) during 24 hr, 12 hr dark cycle and 12 hr light cycle, were measured. Note that mice used in (H)–(K) were 6-week-old littermates and had comparable body weight (ERα$^{lox/lox}$, 16.63 ± 1.01 versus ERα$^{lox/lox}$/SF1-Cre/POMC-Cre, 18.09 ± 0.51, $p > 0.05$) and fat mass (ERα$^{lox/lox}$, 2.15 ± 0.37 versus ERα$^{lox/lox}$/SF1-Cre/POMC-Cre, 1.88 ± 0.26, $p < 0.01$) but different lean mass (ERα$^{lox/lox}$, 12.33 ± 0.35 versus ERα$^{lox/lox}$/SF1-Cre/POMC-Cre, 13.69 ± 0.22, $p < 0.01$).

(L) Plasma norepinephrine and epinephrine in chow-fed females (n = 4/genotype, 6 weeks of age). Data are presented as mean ±SEM, and *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ between ERα$^{lox/lox}$/SF1-Cre/POMC-Cre mice and ERα$^{lox/lox}$ mice.

indicating that ERα is also deleted from these pituitary ACTH cells. However, we did not find any significant changes in POMC (ACTH) expression in the pituitary (Figure S5B). In addition, plasma levels of corticosterone at either basal or stressed conditions were comparable between ERα$^{lox/lox}$/POMC-Cre females and controls (Table S2). Morphologic analysis did not reveal any alterations in the pituitary (Figure S5B). In addition, we did not detect any significant changes in plasma T3/T4 levels (Table S2). Therefore, it is unlikely that the metabolic and reproductive phenotypes observed in ERα$^{lox/lox}$/POMC-Cre females are due to loss of ERα from the pituitary ACTH cells, although we cannot fully rule out this possibility.

Double Deletion of ERα from SF1/POMC Neurons Affects Feeding, Energy Expenditure, and Fat Distribution

Our data indicated that ERα in SF1 neurons is required to regulate energy expenditure and fat distribution, while ERα in POMC neurons is required for the regulation of feeding. To further confirm this model, we generated mice lacking ERα in both SF1 and POMC neurons (ERα$^{lox/lox}$/SF1-Cre/POMC-Cre) and the control (ERα$^{lox/lox}$) littermates.

Chow-fed male ERα$^{lox/lox}$/SF1-Cre/POMC-Cre mice and controls showed comparable body weight and body composition (Figures 7A and 7B). Female ERα$^{lox/lox}$/SF1-Cre/POMC-Cre mice had significantly increased body weight (Figure 7C), which was mainly reflected by increases in lean mass (Figure 7D). Although the whole-body fat mass was not different (Figure 7D), the average weight of gonadal fat was significantly increased in ERα$^{lox/lox}$/SF1-Cre/POMC-Cre females (Figure 7E). Similar to ERα$^{lox/lox}$/SF1-Cre females, subsets of ERα$^{lox/lox}$/SF1-Cre/POMC-Cre females displayed the massive gonadal fat expansion (Figure 7F). While 30% of ERα$^{lox/lox}$/SF1-Cre females developed this phenotype during adulthood (2–3 months), 50% of ERα$^{lox/lox}$/SF1-Cre/POMC-Cre females developed the same phenotype at earlier ages (as early as 2 weeks of age) (Figure 7G).

Further, ERα$^{lox/lox}$/SF1-Cre/POMC-Cre females were not only hyperphagic (Figure 7H) but also showed decreased heat production (Figure 7I). No significant difference was observed in ambulatory movements and rearing activities in female mice (Figures 7J and 7K). Finally, ERα$^{lox/lox}$/SF1-Cre/POMC-Cre females had significantly lower plasma norepinephrine levels than controls; epinephrine levels were not significantly different (Figure 7L). Collectively, these findings indicate that ERα expressed by SF1 and POMC neurons provides the coordinated control of food intake, energy expenditure, and fat distribution.

Cre Transgenes Do Not Affect Body Weight

To rule out the possibility that the Cre transgenes used in the present study may have independently contributed to the metabolic phenotypes, we generated parallel cohorts of transgenic mice carrying only the Nestin-Cre, SF1-Cre, or POMC-Cre transgene and their respective wild-type littermates. These mice were produced at the same genetic background as those conditional knockout mice. No significant change in body weight was observed in these transgenic Cre mice (Figure S6).

DISCUSSION

Genetic Segregation of ERα Functions in the Brain

The estrogen/ERα system is known to regulate food intake, energy expenditure, physical activity (Gao et al., 2007), and fat distribution (Heine et al., 2000). ERα-expressing cells mediating these estrogenic effects were not identified prior to our study. The phenotypic comparison in the four mouse models we generated provides evidence to support a segregation model in which ERα is expressed by distinct hypothalamic neurons and mediates different functions of estrogens in the context of energy homeostasis.

Mice lacking ERα in the CNS have hyperphagia and decreased energy expenditure. These results indicate that CNS ERα is required to suppress food intake and increase energy expenditure. The anorexigenic effects of estrogens are further pinpointed to be mediated by ERα-positive POMC neurons, as mice lacking ERα in POMC neurons or in both POMC and SF1 neurons develop hyperphagia, while deletion of ERα only in SF1 neurons does not affect feeding.

We identify ERα-positive SF1 neurons as the key site where estrogens act to stimulate energy expenditure, since deletion of ERα in SF1 neurons produces hypometabolic phenotypes. The increased energy expenditure seen in ERα$^{lox/lox}$/POMC-Cre mice is unexpected, as estrogens are known to activate POMC neurons (Malyala et al., 2008), and activation of POMC neurons would increase energy expenditure (Morton et al., 2006). The fact that ERα$^{lox/lox}$/POMC-Cre mice have elevated estradiol-17β levels suggests that the increased energy expenditure may result from compensatory estrogenic actions in other ER sites. Since mice lacking ERα in the CNS show decreased energy expenditure despite the elevated estradiol-17β levels, these "compensatory" estrogen signals must be neuronal in origin. In addition, decreased energy expenditure in ERα$^{lox/lox}$/SF1-Cre/POMC-Cre mice further demonstrates that ERα-positive SF1 neurons are at least one site where elevated estradiol-17β acts to stimulate energy expenditure in ERα$^{lox/lox}$/POMC-Cre mice.

Estrogen/ERα signals also suppress fat accumulation in the visceral adipose depot (Heine et al., 2000; Rogers et al., 2009). We show that CNS deletion of ERα leads to increased visceral fat distribution. Further, mice lacking ERα in SF1 neurons or in both SF1 and POMC neurons show increased visceral fat distribution, whereas deletion of ERα in POMC neurons alone does not influence fat distribution. Therefore, these findings indicate that ERα in SF1 neurons is required to mediate estrogenic effects on fat distribution.

Collectively, our results indicate that estrogenic effects on food intake, energy expenditure, and fat distribution are mediated by segregated hypothalamic ERα populations. Thus, ERα in SF1 neurons is required to maintain normal energy expenditure and fat distribution, while ERα in POMC neurons regulates feeding.

Notably, CNS deletion of ERα leads to hypoactivity, indicating that CNS ERα is required to stimulate physical activity. However, this phenotype is not observed in mice lacking ERα in SF1 neurons, in POMC neurons, or in both, which implies that other CNS neurons expressing ERα may be responsible for these effects. Of note, animals with ERα knocked down in the VMH display decreased physical activity (Musatov et al., 2007). Together with our observations, these findings suggest that non-SF1 neurons in the VMH may mediate estrogenic actions to regulate physical activity.

ERα mutations in male mice and men cause obesity (Heine et al., 2000; Smith et al., 1994). Consistently, we show that CNS deletion of ERα also promotes body weight gain in males. These findings highlight the physiological relevance of ERα in male brains in the regulation of energy balance. Notably, deletion of ERα in SF1 neurons, POMC neurons, or both does not affect body weight in male mice. Collectively, these findings indicate that other ERα sites in male brains contribute to the regulation of energy balance. Further efforts are warranted to unravel these unknown ERα sites in male brains.

Estrogenic Actions in VMH SF1 Neurons

The major metabolic phenotypes in mice lacking ERα in SF1 neurons include decreased energy expenditure (e.g., BAT thermogenesis) and increased lipid accumulation in gonadal WAT. Both these deficits could be attributed to decreased sympathetic tone (lower norepinephrine). Consistently, electric stimulation of the VMH increases sympathetic inputs to BAT and enhances thermogenesis (Saito et al., 1987). Lipid metabolism in gonadal WAT is also regulated by the sympathetic nervous system. For example, increased lipolysis in gonadal WAT is associated with elevated sympathetic inputs (Plum et al., 2007), while the expanded gonadal WAT is found to have decreased sympathetic inputs (Ramadori et al., 2010). Collectively, these findings support a model in which estrogens act on ERα in VMH SF1 neurons to increase central sympathetic outflow, which leads to increased BAT thermogenesis and inhibits lipid storage in gonadal WAT.

The intracellular mechanisms by which estrogen/ERα regulate SF1 neurons are not fully understood. Park et al. reported that estrogens activate the rapid PI3K-Akt pathway in the VMH via an ERα-dependent mechanism (Park et al., 2011). Remarkably, restoration of ERα-associated rapid signaling pathways (including the PI3K-Akt pathway) at the global ERα null

background is sufficient to rescue obesity (Park et al., 2011). In addition, we have previously shown that selective inhibition of PI3K in SF1 neurons leads to obesity (Xu et al., 2010). Collectively, these findings suggest that the PI3K pathway (and/or other rapid signaling pathways) in SF1 neurons may mediate antiobesity effects of estrogens.

Alternatively, ERα may regulate SF1 neurons as a transcription factor. We show that mice lacking ERα in SF1 neurons have decreased expression of leptin receptors in the VMH. Consistent with a possible role of estrogen/ERα on expression of leptin receptors, these two receptors are found to be coexpressed by VMH neurons (Diano et al., 1998), and an estrogen response element (ERE) was found in the promoter region of the leptin receptor gene (Lindell et al., 2001). In addition, selective deletion of leptin receptors in SF1 neurons produces similar obese phenotypes (Dhillon et al., 2006). Collectively, these data suggest that ERα in SF1 neurons may regulate energy homeostasis, at least partly, by modulating leptin sensitivity in the VMH.

Estrogenic Actions in POMC Neurons

Mice lacking ERα in POMC neurons develop hyperphagia, indicating that ERα signals in POMC neurons are physiologically relevant in the regulation of feeding. This regulation may be mediated by estrogenic actions on POMC neural activity, as estrogens have been shown to activate POMC neurons (Gao et al., 2007; Malyala et al., 2008). Alternatively, estrogens may regulate feeding partly via sensitizing responses to other anorexigenic hormones, such as leptin. We previously demonstrated that estrogens potentiate the anorexigenic effects of leptin (Clegg et al., 2006). We now extend those findings to demonstrate that the effects of leptin on food intake are blunted in mice lacking ERα in POMC neurons. Importantly, the efficacy of MTII's anorexia is not affected in these mice, suggesting that the impairments of estrogenic actions are constrained to the POMC neurons. Recent evidence indicates that estrogens, similar to leptin, induce STAT3 phosphorylation in the hypothalamus, and that the antiobesity effects of estrogens are abolished in mice lacking STAT3 in the brain (Gao et al., 2007). Therefore, it is possible that estrogens may initiate signals in POMC neurons which ultimately converge on the leptin-induced pSTAT pathway to regulate food intake.

While the majority of POMC neurons reside in the ARH of the hypothalamus, a small subset of POMC neurons are also found in the NTS of the brain stem, which are implicated in the regulation of satiety (Fan et al., 2004). In addition, estrogens increase c-*fos* immunoreactivity in the NTS via ERα-mediated mechanisms (Asarian and Geary, 2007). Notably, our POMC-Cre induced efficient ERα deletion in the NTS, as well as in the ARH. Therefore, these findings support an alternative model in which estrogens may regulate food intake at least partly by acting on ERα expressed by NTS POMC neurons.

Hypothalamic ERα and Reproduction

$ER\alpha^{lox/lox}$/SF1-Cre females develop infertility likely due to anovulation (the lack of ovarian corpus luteum). An important question remains as to whether this ovarian phenotype results from loss of ERα in VMH SF1 neurons or from loss of ERα in ovarian SF1 cells. Although we did not find significant reduction of ERα mRNA in the ovary of $ER\alpha^{lox/lox}$/SF1-Cre mice, we could not fully exclude the possibility that ERα is deleted in ovarian SF1 cells. On the other hand, it is interesting to note that our $ER\alpha^{lox/lox}$/SF1-Cre female mice recapitulate the ovarian phenotypes (numerous antral follicles and lack of corpus luteum) seen in $ER\alpha^{lox/lox}$/CAMKII-Cre mice, a brain-specific ERα knockout model (Wintermantel et al., 2006). Therefore, it is possible that the ovarian phenotypes are caused by loss of ERα in SF1 neurons in the brain. Supporting this notion, genetic ablation of SF1 neurons in the brain leads to decreased or reduced corpora luteum and subfertility/infertility in female mice (Kim et al., 2010).

Deletion of ERα in POMC neurons impaired negative feedback regulation of estrogens on the HPG axis. The negative feedback is primarily mediated by ERα (Couse et al., 2003). ERα in pituitary LH cells partially mediates this effect, as deletion of ERα in LH cells blunts estrogen-induced inhibition on LH secretion (Singh et al., 2009). Alternatively, estrogens may act on gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus to inhibit the HPG axis. As GnRH neurons do not express ERα (Shivers et al., 1983), interneurons must exist to relay the negative feedback signals to GnRH neurons. Our observations that the negative feedback is impaired in $ER\alpha^{lox/lox}$/POMC-Cre females indicate that POMC neurons may be one of these interneurons. Supporting this notion, ARH POMC neurons project to the rostral preoptic area, where GnRH neurons are concentrated (Simonian et al., 1999). The possible signals that originate from POMC neurons and synapse on GnRH neurons are unclear. Recent studies from lean hypogonadotropic ewes suggested that α-MSH mediates leptin effects to restore pulsatile LH secretion (Backholer et al., 2010). Additionally, estrogens are implicated to increase the secretion of another POMC gene product, β-endorphin (Lagrange et al., 1994), which inhibits GnRH neurons (Lagrange et al., 1995). These findings support a model in which estrogens act on ERα in POMC neurons to modulate secretion of POMC peptides (e.g., α-MSH and/or β-endorphin), which in turn act on GnRH neurons to mediate the negative feedback on the HPG axis.

Other ERα populations in the brain may also contribute to the negative feedback regulation. For example, Kiss1 neurons are required for the tonic stimulation of GnRH/LH secretion (Dungan et al., 2007). Interestingly, Kiss1 neurons in the ARH coexpress ERα (Cravo et al., 2011), and estrogens reduce ARH Kiss1 expression (Smith et al., 2005). Of note, Kiss1 neurons and POMC neurons are exclusively segregated in the ARH (Cravo et al., 2011). Therefore, it is possible that ERα in ARH Kiss1 neurons may provide an alternative pathway, in addition to ERα in POMC neurons, to mediate the negative feedback.

Conclusions

Using mice lacking ERα in the CNS, in SF1 neurons, in POMC neurons, and in both SF1 and POMC neurons, we have substantially narrowed down the critical ERα sites that mediate the estrogenic effects on energy homeostasis and reproduction. These results provide genetic evidence to segregate physiological functions of ERα cells in the context of obesity and infertility, and may provide rational targets for the development of highly selective hormone replacement therapies which may be used to combat obesity and infertility in women.

EXPERIMENTAL PROCEDURES

Animals

Care of all animals and procedures were approved by the University of Cincinnati, University of Texas Southwestern Medical Center, and Baylor College of Medicine. Mice were housed in a temperature-controlled environment in groups of two to five at 22°C–24°C using a 12 hr light/12 hr dark cycle. Some cohorts were singly housed to measure food intake. The mice were fed either standard phytoestrogen-free chow (#2916, Harlan-Teklad, Madison, WI) or 42% HFD (#88137, Harlan Teklad), and water was provided ad libitum.

Body Weight, Food Intake, and Body Composition

Body weight was measured weekly. Food intake was measured daily, and average daily food intake was calculated using data from at least four continuous days (7 day data were used in the 5-week-old $ER\alpha^{lox/lox}$/SF1-Cre group). Body composition was determined using quantitative magnetic resonance (QMR).

Assessment of Negative Feedback

Twelve-week-old chow-fed female $ER\alpha^{lox/lox}$/POMC-Cre mice and their $ER\alpha^{lox/lox}$ controls were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and received bilateral ovriectomy, followed by subcutaneous implantations of pellets containing estradiol-17β (0.5 μg/day/mouse with 60 day releasing capacity, OVX+E) or containing vehicle (OVX+V). Four weeks later, these mice were sacrificed in the afternoon (between 1 and 4 p.m.) after deep anesthesia. The pituitary was quickly isolated; trunk blood was collected and processed to collect plasma. Expression of FSH and LH in the pituitary and plasma FSH/LH were analyzed as described in the Supplemental Experimental Procedures.

Statistics

The data are presented as mean ±SEM. Statistical analyses were performed using SigmaStat 2.03. After confirming normal distribution of data, comparisons between two genotypes were made by the unpaired two-tailed Student's t test; repeated measures ANOVA was used to compare changes over time between two genotypes. Two-way ANOVA analyses were used to assess the interactions between genotypes and treatments (e.g., saline versus drugs). $p < 0.05$ was considered to be statistically significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures, two tables, Supplemental Experimental Procedures, and Supplemental References and can be found with this article online at doi:10.1016/j.cmet.2011.08.009.

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