1521-0103/371/1/113–120$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

https://doi.org/10.1124/jpet.118.255380
J Pharmacol Exp Ther 371:113–120, October 2019

The Journal of PHARMACOLOGY And Experimental Therapeutics

Effect of Pregnancy on Paroxetine-Induced Adiposity and Glucose Intolerance in Mice

Weibin Zha,[1] Tao Hu, Mary F. Hebert, and Joanne Wang

Departments of Pharmaceutics (W.Z., T.H., J.W.), Pharmacy (M.F.H.), and Obstetrics and Gynecology (M.F.H.), University of Washington, Seattle, Washington

Received November 30, 2018; accepted July 11, 2019

ABSTRACT

Long-term use of selective serotonin reuptake inhibitors (SSRIs) targeting the serotonin transporter (SERT) has been suggested to be associated with an increased risk for obesity and type 2 diabetes. Previously, using a murine knockout model of SERT, we showed that estrogen suppression is involved in SERT deficiency–induced obesity and glucose intolerance in nonpregnant mice. The present study investigated the effects of chronic paroxetine treatment on adiposity and glucose tolerance in mice before and during pregnancy. Chronic paroxetine treatment in nonpregnant mice resulted in visceral adiposity and glucose intolerance accompanied by reduced circulating 17β-estradiol levels and ovarian expression of the aromatase (CYP19a1). Remarkably, pregnancy significantly reduced adiposity and improved glucose tolerance in paroxetine-treated mice by rebooting ovarian CYP19a1 expression and 17β-estradiol production. These effects appear to be reversible as ovarian CYP19a1 expression and circulating 17β-estradiol returned to prepregnancy levels soon after parturition. As in pregnant mice, 17β-estradiol replacement treatment in nonpregnant mice reduced paroxetine-induced adiposity. Our findings further suggested that modulation of estrogen synthesis underlies the observed metabolic adverse effects of SSRIs. Although our data revealed a transient reversal effect of pregnancy on SSRI-induced metabolic abnormalities, these observations are experimental and limited to mice. The use of SSRIs during human pregnancy should be cautioned because of potential adverse effects to the fetuses.

Introduction

The serotonin transporter (SERT) is a key regulator of the serotoninergic system and a major target of antidepressants. The selective serotonin reuptake inhibitors (SSRIs), which specifically inhibit SERT, are currently the most widely prescribed class of antidepressants in the world. The risk of major depression is high among women during the childbearing years with an estimated prevalence of up to 10%–20% (Steiner, 1998; Marcus et al., 2003; Barbour, 2014). Because of the harmful consequences of untreated depression, including increased risk for maternal and neonatal morbidities, antidepressants, including SSRIs, remain an option during pregnancy (Cooper et al., 2007; Andrade et al., 2008); however, SSRI use during pregnancy has been associated with a number of potential complications for neonates, including low birth weight, congenial abnormality, pulmonary hypertension, and neurodevelopmental problems (Alwan and Friedman, 2009; Toh et al., 2009; Lindqvist et al., 2014). The risks and benefits of treatment should be carefully evaluated when deciding whether to use SSRIs during pregnancy.

In the general population of depression, chronic use of SSRIs, such as paroxetine, is associated with weight gain and an increased risk of type 2 diabetes (McIntyre et al., 2006; Raeder et al., 2006; Andersohn et al., 2009; Serretti and Mandelli, 2010; Hennings et al., 2012). Mice or rats with targeted deletion of the SERT gene (*Slc6a4*) developed obesity, insulin resistance, and glucose intolerance without increasing food intake, suggesting that these effects are not due to changes in appetite control (Homberg et al., 2010; Chen et al., 2012). Nevertheless, preclinical or clinical studies have not been performed to evaluate the effect of SSRIs on fat and glucose metabolism during pregnancy, a state where insulin resistance has already been imposed to allow nutrient flow to the fetus (Lain and Catalano, 2007). Although there is currently little clinical evidence to suggest a link between SSRI use and increased risk for gestational diabetes mellitus, the obesogenic and diabetogenic potential of SSRIs remains a significant concern for pregnant women (Kulkarni et al., 2015).

Using SERT knockout (KO) mice, we recently showed that SERT deletion led to abnormal fat accumulation, insulin resistance, and glucose intolerance in nonpregnant mice through suppressing aromatase (CYP19a1) expression and

This work was supported in part by National Institute of Health Grants R01 GM066233 (to J.W.), U10 HD047892 (to M.F.H.), and funding from the Elmer M. Plein Endowment Research Fund (to W.Z.).
[1]Current affiliation: MyoKardia, South San Francisco, CA 94080.
https://doi.org/10.1124/jpet.118.255380.

ABBREVIATIONS: ACC1, acetyl-CoA carboxylase 1; ADRβ3, β3-adrenergic receptors; ANOVA, analysis of variance; BAT, brown adipose tissue; 17β-E, 17β-estradiol; FAS, fatty acid synthase; GD, gestational day; 5-HT, 5-hydroxytryptamine (serotonin); KO, knockout; LPL, lipoprotein lipase; NE, norepomephrine; PD, postpartum day; SERT, serotonin transporter; SNS, sympathetic nervous system; SSRIs, selective serotonin reuptake inhibitors; UCP1, uncoupling protein 1; WAT, white adipose tissue; WT, wild- type.

reducing circulating estrogen levels (Zha et al., 2017). Interestingly, pregnancy normalized fat accumulation and improved insulin sensitivity and glucose response in the SERT KO mice by restoring CYP19a1 expression and estrogen levels in these animals; however, these studies were conducted in mice with the inborn deletion of SERT. Although SSRI treatment of nonpregnant wild-type (WT) mice also resulted in obesity and glucose intolerance via CYP19a1 and estrogen suppression (Zha et al., 2017), it is unknown how these SSRI-associated adverse effects are impacted by pregnancy. Further, the effect of SSRIs on lipogenesis, CYP19a1 expression, and estrogen levels has never been examined during the postpartum period. The goal of this study is to evaluate the impact of SSRI use on adipose tissue deposit, lipogenesis, CYP19a1 expression, and circulating estrogen levels before, during, and after pregnancy to explore more fully the role of estrogen in SSRI-induced obesity and glucose intolerance in mice.

Materials and Methods

Animals. All mice used in the experiments were bred on a C57BL/6 background and housed in specific-pathogen-free facility at the University of Washington. Mice had free access to a standard diet (PicoLab Rodent diet 20 no. 5053; LabDiet, St. Louis, MO) and water and were housed in controlled conditions for temperature and humidity using a 12-hour light/dark cycle. All animal studies were approved by the Institutional Animal Care and Use Committee of the University of Washington.

Paroxetine and Estrogen Treatment. From weaning at the age of 3 weeks onward, WT female mice were treated with paroxetine hydrochloride (10 mg/kg per day; Ark Pharma, Inc, Libertyville, IL) administered in the drinking water for 12 weeks. Drinking solution (50 mg/liter, paroxetine in water) was prepared fresh every week. Body weight was monitored weekly. Female mice that had been treated with or without paroxetine for 12 weeks were mated, with vaginal plug date assigned as gestational day (GD) 0. Body weight was measured on GD 0, 8, 13, and 18 of pregnancy and postpartum day (PD) 1. Nonpregnant mice that were treated with or without paroxetine for 12 weeks were implanted subcutaneously with a 17β-estradiol (17β-E) pellet that delivered 2.5 μg of 17β-E daily or a placebo pellet (Innovative Research of America, Sarasota, FL) under isoflurane anesthesia (initial induction 4%, maintenance 1.5%). 17β-E-treated mice were monitored for a total of 3 weeks. At the end of the experiments, nonpregnant and pregnant mice at GD 14 and PD 1 were euthanized by CO_2 inhalation. Blood was collected via cardiac puncture, and plasma was separated by centrifugation. Fat pads (gonadal, inguinal, retroperitoneal, and brown adipose), ovaries, fetuses, and placentas were harvested and weighed, with part of the tissue snap-frozen in liquid N_2 and the remainder fixed in 10% formalin and embedded in paraffin for histologic analysis.

RNA Isolation and Quantitative Polymerase Chain Reaction. Total RNA was extracted from white adipose tissue (WAT), brown adipose tissue (BAT), and ovaries using the RNeasy Miniprep kit (Qiagen, Valencia, CA). Total RNA (2 μg) was reverse-transcribed to cDNA, as described by others (Lee et al., 2013; Zha et al., 2017). Expression of murine lipoprotein lipase (*LPL*) (Mm00434764_m1), acetyl-CoA carboxylase 1 (*ACC1*) (Mm01304257_m1), fatty acid synthase (*FAS*) (Mm00662319_m1), uncoupling protein 1 (*UCP1*) (Mm01244861_m1), *Cyp19a1* (Mm00484049_m1), and *GAPDH* (Mm99999915_g1) was quantified using TaqMan Assays (Life Technologies, Carlsbad, CA) as described previously (Duan and Wang, 2010; Zha et al., 2017). Gene expression was normalized to GAPDH expressed relative to control using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Adipose Histology. WAT and BAT were processed to paraffin-embedded sections (5-μm sections, 100 μm apart) and then stained with H&E and scanned into digital images (ScanScope CS; Aperio, Vista, CA). WAT adipocyte size per 10× field and BAT lipid droplets size and number per 20× field were quantified using NIH ImageJ software and the magnetic resonance imaging adipocyte tool, as described (Zha et al., 2014; McGlashon et al., 2015; Zha et al., 2017). An average value across nine nonoverlapping fields (three fields/section × three sections/mouse) was calculated for each mouse.

Glucose Tolerance Tests. Glucose tolerance test (2 g/kg body weight, injected intraperitoneally) was performed in nonpregnant and pregnant (GD 14) mice after a 16-hour overnight fast as previously described (Zha et al., 2017). Whole-blood glucose concentrations were measured before and 15, 30, 60, 90, and 120 minutes after dosing with a One-Touch Ultra Glucometer (LifeScan, Milpitas, CA). The area under the glucose concentration-time curve (glucose $AUC_{0-120\ min}$) was calculated using the trapezoidal method.

Immunoblotting. Protein was extracted from ovaries using RIPA buffer containing 50 mM Tris-HCl (pH 8.0), 1% Triton × 100, 150 mM NaCl, 1 mM EDTA, 0.25% sodium deoxycholate, 1 mM sodium fluoride, 1 mM sodium or thovanadate, and protease inhibitor mixture (Roche Applied Science, Indianapolis, IN). Protein concentrations were determined with a Pierce BCA Protein Assay Kit (Thermo Scientific, Waltham, MA). Protein samples were subjected to SDS-PAGE and then transferred to polyvinylidene fluoride membranes (EMD Millipore Corporation, Burlington, MA). The blot was incubated with anti-CYP19a1 polyclonal antibody (sc-14245, 1:500; Santa Cruz Biotechnology) and followed by detection with a horseradish peroxidase-conjugated mouse anti-goat IgG (sc-2354, 1:4000; Santa Cruz Biotechnology, Dallas, TX). Immunoreactive bands were detected by chemiluminescence using the enhanced chemoluminescence Western blotting substrate (Thermo Scientific). The density of the immunoreactive bands was analyzed using NIH ImageJ Software.

Enzyme-Linked Immunosorbent Assay. Plasma17β-E were measured using a commercial mouse enzyme-linked immunosorbent assay kit (Calbiotech, San Diego, CA) as previously described (Zha et al., 2017).

Statistical Analysis. All data are presented as mean ± S.E.M. Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA). Normal distribution was tested by the *f*-test. Two-tailed Student's *t* test was used to analyze the difference across two groups, and one-way analysis of variance (ANOVA) with a post hoc Dunnett's test was used to determine the difference between multiple experimental groups when data were normally distributed. One-way ANOVA with a post hoc Wilcoxon rank-sum test was used when data were not normally distributed. Differences in the glucose concentration-time profile were determined using two-way repeated-measures ANOVA and Bonferroni's post-hoc tests. $P < 0.05$ was considered significant.

Results

Effect of Paroxetine on Body Weight before and during Pregnancy. Female C57BL/6 mice were treated with or without paroxetine (10 mg/kg per day) for 12 weeks after weaning. This dose of paroxetine is known to achieve serum concentrations in mice comparable to the therapeutic levels observed in humans (Christensen et al., 1998; Hiemke and Hartter, 2000; Tang and Helmeste, 2008). Compared with the vehicle control, paroxetine-treated mice started to gain more weight after entering adulthood (Fig. 1A), showing an ~12% increase in body weight at the age of 12–15 weeks (Fig. 1A). During pregnancy, total body weight increased progressively in both control and treated groups (Fig. 1B); however, we found no significant difference between treated

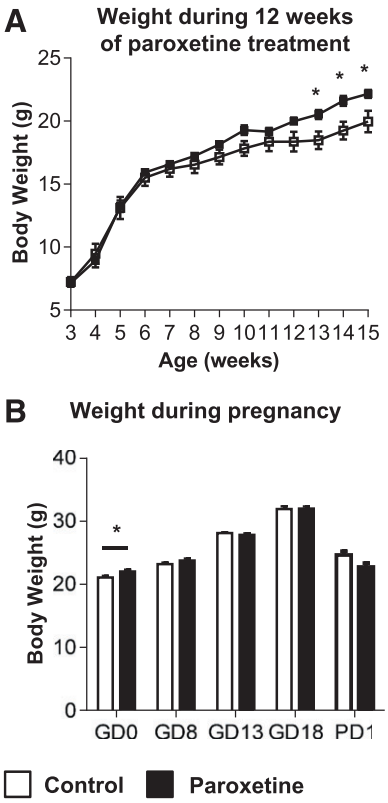

Fig. 1. Effects of paroxetine on body weight and food intake in nonpregnant and pregnant mice. (A) Body weights of wild-type (WT) female mice treated with or without paroxetine (10 mg/kg per day) for 12 weeks ($n = 5$ per group). (B) Body weights of WT female mice treated with or without paroxetine after 12 weeks at gestation day (GD) 0, 8, 13, 18, and postpartum day (PD) 1 ($n = 5$ per group). *$P < 0.05$. Values are the mean ± S.E.M.

and untreated pregnant mice at GD 8, 13, and 18 (Fig. 1B). Thus, pregnancy normalized the body weight difference between paroxetine-treated and nontreated mice observed before pregnancy.

Effect of Pregnancy on Paroxetine-Induced Adiposity. Consistent with our previous findings (Zha et al., 2017), we observed a significant increase in gonadal, inguinal, and retroperitoneal white-fat pads in paroxetine-treated mice before pregnancy (Fig. 2, A–C). Pregnancy significantly reduced gonadal and retroperitoneal fat-pad weight in paroxetine-treated mice (Fig. 2, A–C). Consistent with white-fat weight changes, pregnancy attenuated lipid storage in gonadal WAT in pregnant paroxetine-treated mice (Fig. 2, D and E). In contrast, BAT mass increased during pregnancy but decreased to prepregnancy levels after delivery independent of paroxetine treatment (Fig. 2F). Histologic analysis showed that BAT from paroxetine-treated mice appeared to contain a greater amount of unilocular fat droplets before pregnancy (Fig. 2G); however, pregnancy significantly reduced unilocular lipid droplets in paroxetine-treated mice (Fig. 2G). This finding was further confirmed by analysis of lipid droplet area and number, showing a reduction in lipid droplet size and an increase of lipid droplet number in paroxetine-treated mice at GD 14 and PD 1 compared with paroxetine-treated nonpregnant mice (Fig. 2, H and I). Expression of genes involved in lipogensis in gonadal WAT (GWAT) and thermogenesis in BAT was further determined and compared in paroxetine-treated mice before pregnancy and at GD 14 and PD 1. The lipogenic markers FAS, ACC1, and LPL in gWAT in paroxetine-treated mice showed significant decreases in mRNA expression during pregnancy but were immediately restored to prepregnancy levels at PD 1 (Fig. 3, A–C). Alteration of lipid accumulation in BAT is often accompanied by changes in UCP1 expression, which is a key regulator of thermogenesis (Wu et al., 2013). Consistent with less lipid accumulation in BAT during pregnancy and at PD 1 in paroxetine-treated mice (Fig. 2G), UCP1 mRNA levels in paroxetine-treated mice were increased during pregnancy and remained elevated at PD 1 (Fig. 3D).

Effect of Paroxetine Treatment on Glucose Tolerance before and during Pregnancy. Obesity is a well established causal factor in mediating insulin resistance and glucose intolerance (Kahn and Flier, 2000). Consistent with paroxetine-induced obesity (Figs. 2 and 3), paroxetine-treated nonpregnant mice exhibited increased fasting blood glucose levels (Fig. 4A; time 0) and decreased glucose tolerance (Fig. 4, A and C) compared with nontreated controls. Pregnancy is known to induce insulin resistance in the mother, which is important for maintaining a normal nutrient flow to the growing fetus (Lain and Catalano, 2007). As expected, untreated pregnant mice showed a significant induction of systemic glucose intolerance compared with untreated nonpregnant mice (Fig. 4C). In contrast, paroxetine-treated mice showed no further deterioration in glucose tolerance during pregnancy and even demonstrated better glucose control than untreated pregnant mice (Fig. 4, B and C), which could be due to a sensitized estrogen response after paroxetine-induced estrogen suppression. The reversal in glucose tolerance of these two groups by pregnancy is similar to our previous findings in the SERT KO mice and suggested that pregnancy significantly improved maternal glucose control in the SSRI-treated cohort.

Effect of Paroxetine Treatment on Circulating 17β-E Levels and Ovarian CYP19a1 Expression before, during, and after Pregnancy. Using SERT KO mice, we previously demonstrated that suppression of estrogen synthesis through aromatase (CYP19a1) downregulation is responsible for the obesity and glucose intolerance observed in these KO animals (Zha et al., 2017). Pregnancy can alleviate these metabolic abnormalities in SERT KO mice by normalizing circulating estrogen levels through a SERT-independent pathway (Zha et al., 2017). To determine whether an altered 17β-E level is also responsible for the metabolic changes observed in paroxetine-treated animals, we measured circulating 17β-E levels in control and paroxetine-treated mice before, during, and after pregnancy. Before pregnancy, paroxetine-treated mice exhibited significantly lower circulating 17β-E levels than did untreated mice (Fig. 5A). Pregnancy significantly increased circulating 17β-E levels in both treated and untreated groups (Fig. 5A). After delivery, circulating 17β-E levels declined to prepregnancy levels at PD 1, and paroxetine-treated mice showed significantly lower 17β-E levels than the untreated group (Fig. 5A). Because the rate-limiting step in 17β-E synthesis is mediated by aromatase (CYP19a1) expressed in the ovarian granulosa cells, we examined ovarian CYP19a1 expression in control and treated mice before, during, and after pregnancy. Consistent with the changes in plasma 17β-E levels, paroxetine treatment strongly reduced ovarian CYP19a1 mRNA and protein

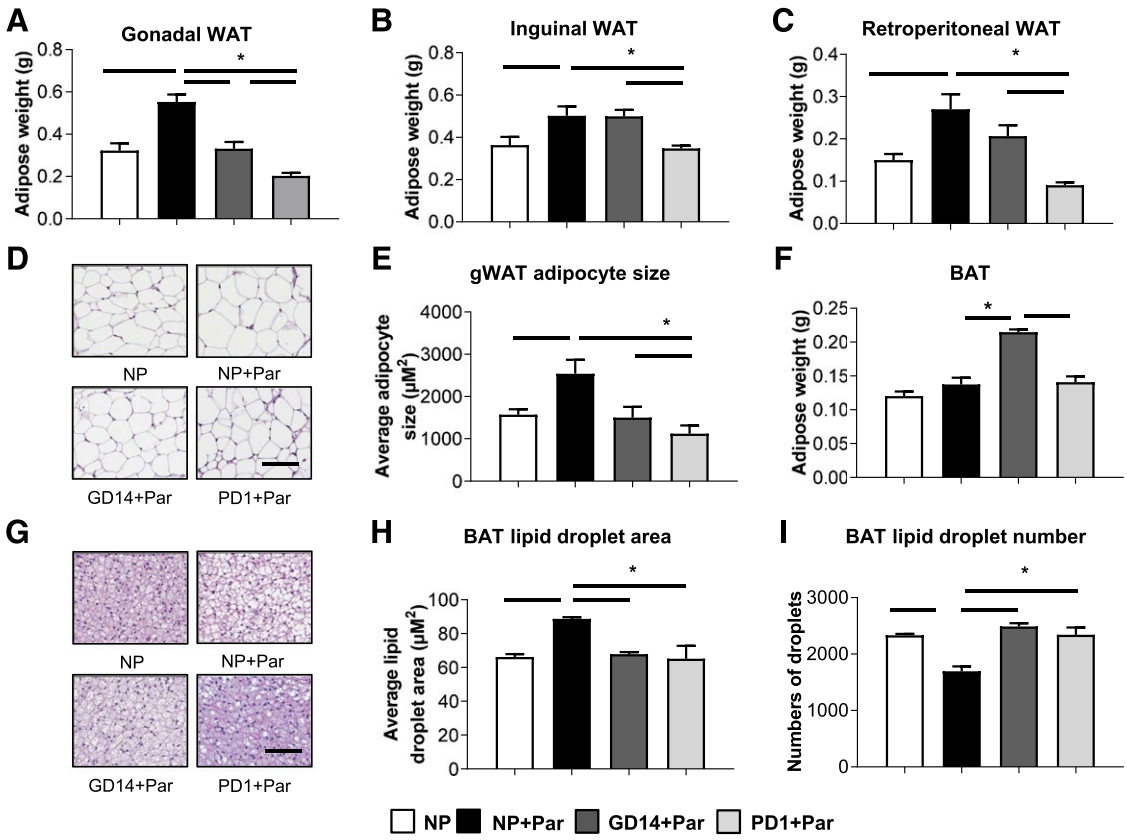

Fig. 2. Effect of pregnancy on paroxetine-induced adiposity. Gonadal (A), inguinal (B), retroperitoneal (C) WAT and (F) BAT weights from control WT nonpregnant mice and WT mice at nonpregnant stage (NP), GD 14, and PD 1 after 12-week treatment with paroxetine (10 mg/kg per day) (n = 5 per group). (D and G) Representative images of H&E-stained gWAT and BAT sections (scale bar, 100 μm). (E, H, and I) Average adipocyte size of gWAT per 10× field and average lipid droplet area and number of BAT per 20× field were quantified using NIH ImageJ. *$P < 0.05$. Values are reported as mean ± S.E.M.

expression in nonpregnant mice (Fig. 5, B and C). Remarkably, pregnancy greatly increased ovarian CYP19a1 mRNA expression in both treated and untreated groups, leading to comparable expression levels of CYP19a1 protein at GD 14 (Fig. 5, B and C). Soon after pregnancy at PD 1, ovarian CYP19a1 expression declined to prepregnancy level in both groups, and the mRNA and protein levels of CYP19a1 at PD1 were significantly lower in the paroxetine-treated group than in the untreated group. Taken together, these results showed that pregnancy can abrogate estrogen suppression imposed by paroxetine treatment in mice, leading to normalized fat metabolism and glucose control in the treated animals. The effects of pregnancy on paroxetine are reversible, and the treated mice returned to a low estrogen state soon after the pups were born.

Estrogen Replacement Reversal of Paroxetine-Induced Adiposity. To further confirm the role of estrogen suppression in paroxetine-induced obesity in the nonpregnant state, we tested whether restoring estrogen levels in paroxetine-treated nonpregnant mice can reduce the abnormal fat mass in these animals. We implanted slow-release 17β-E or placebo pellets into female mice after 12 weeks of paroxetine treatment. Although estrogen replacement did not have a significant effect on total body weight in either paroxetine-treated or untreated mice (Fig. 6A), it effectively attenuated paroxetine-evoked WAT expansion (Fig. 6, B–D). Furthermore, adipocyte hypertrophy (Fig. 6, E and F) and expression of LPL, FAS, and ACC1 in gonadal WAT (Fig. 6G) were significantly attenuated by 17β-E replacement in paroxetine-treated mice. BAT mass and expression of UCP-1 were not significantly changed by 17β-E replacement in paroxetine-treated mice (Fig. 6, H and I) possibly because the level of 17β-E achieved in the estrogen replacement study is not comparable to that gained during normal pregnancy. Alternatively, other pregnancy-related hormones may be involved in regulating BAT function during pregnancy.

Discussion

The goal of this study was to evaluate the impact of SSRI use on adipose tissue depot, lipogenesis, CYP19a1 expression and circulating estrogen levels before, during, and after pregnancy in mice. Our data suggest that 1) pregnancy significantly reduces paroxetine-induced adiposity and glucose intolerance in mice through inhibition of lipid accumulation in both WAT and BAT; 2) the effects of pregnancy on paroxetine-induced metabolic defects are due to a normalization of CYP19a1 expression and estrogen production in the treated animals; 3) the normalizing effect of pregnancy on paroxetine-induced CYP19a1 and estrogen suppression is quickly lost after birth; and 4) estrogen replacement protects nonpregnant mice against paroxetine-induced adiposity. Although our data revealed a transit reversal effect of pregnancy on SSRI-induced metabolic abnormalities in

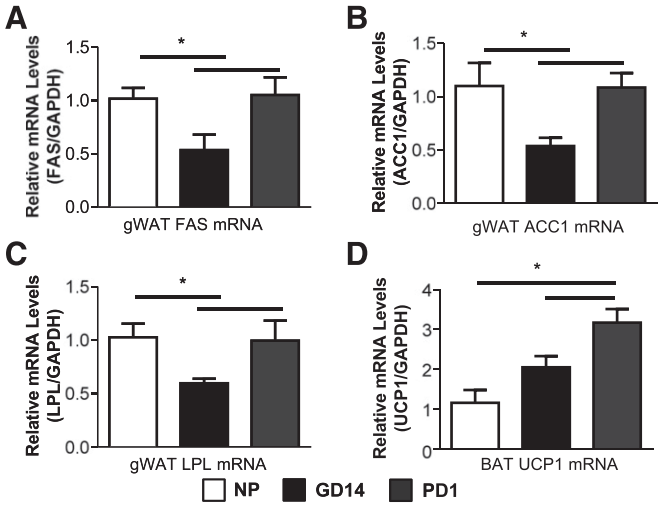

Fig. 3. mRNAs expression of genes involved in lipogenesis in gWAT and thermogenesis in BAT of paroxetine-treated mice. gWAT mRNA levels of LPL (A), FAS (B), ACC1 (C), and BAT mRNA levels of UCP1 (D) from WT mice at NP, GD 14, and PD 1 after 12-week treatment with paroxetine (10 mg/kg per day) were quantified by reverse transcription-quantitative polymerase chain reaction, normalized to GAPDH, and expressed relative to the nonpregnant paroxetine-treated group. $^*P < 0.05$. Values are the mean ± S.E.M.

mice, these observations are experimental and not applicable to human pregnancy. The use of SSRIs during human pregnancy should be cautioned because of potential adverse effects to the fetuses.

Paroxetine is a frequently prescribed SSRI that has been reported to have side effects on fat and glucose metabolism in the general population (Raeder et al., 2006; Derijks et al., 2008; Serretti and Mandelli, 2010). We used paroxetine to test the effect of SSRIs on fat and glucose metabolism during pregnancy as its use is reportedly associated with significant weight gain in the general population of depression (Fava et al., 2000; Raeder et al., 2006). Indeed, our results showed that before pregnancy, paroxetine-treated mice gained more weight after entering adulthood (Fig. 1). These treated animals showed increased fat mass and worsened glucose control (Figs. 2–4). Pregnancy significantly reduced fat mass and improved glucose intolerance in paroxetine-treated mice (Figs. 2–4), which is consistent with our previous observation in pregnant SERT KO mice (Zha et al., 2017). Moreover, we observed a pregnancy-related increase in circulating 17β-E in both treated and untreated mice, accompanied by a dramatic increase in ovarian CYP19a1 expression (Fig. 5). As estrogen is known to exert potent antiobesity and antidiabetic effects in both nonpregnant and pregnant status (Riant et al., 2009; Stubbins et al., 2012; Pedroni et al., 2014), our results suggest that pregnancy-evoked estrogen surge plays a fundamental role against paroxetine-induced adiposity and glucose intolerance in mice. Indeed, implantation of 17β-E pellets significantly reduced fat mass in nonpregnant mice treated with paroxetine compared with placebo-implanted controls (Fig. 6). Collectively, our data showed that chronic treatment of SSRI in mice suppresses CYP19a1 expression and circulating estrogen levels, leading to increased propensity for adiposity and glucose intolerance in nonpregnant mice (visual abstract). Pregnancy abolishes SSRI-induced ovarian CYP19a1 suppression and replenishes circulating estrogen levels; thus, it reverses adiposity and glucose intolerance in treated animals (visual abstract).

All SSRIs exert their pharmacologic effects by specifically inhibiting SERT. A limitation of the present study is that we performed our study with only one SSRI drug, paroxetine. Although paroxetine was historically prescribed to pregnant women (Andrade et al., 2008), the U.S. Food and Drug Administration has recommended avoiding paroxetine use during pregnancy because of the risk of congenital cardiovascular abnormality for newborns (Berard et al., 2012; Berard et al., 2016). The consensus findings between the present paroxetine study and our previous work in the SERT (the therapeutic target of all SSRIs) knockout mice suggest that the observed effects of paroxetine during nonpregnant and pregnant states are likely applicable to other SSRIs. Nevertheless, comprehensive evaluation of the specific impact of other SSRIs on lipid and glucose metabolism in nonpregnant and pregnant states remains necessary for understanding the clinical implications of SSRIs commonly used during pregnancy. In addition, it is important to note that our study is not intended to evaluate the safety and toxicities of SSRIs during pregnancy. Clinically, the risks and benefits of treatment should be carefully evaluated in deciding whether to use SSRIs during pregnancy.

Another significant finding of our study was that pregnancy could reverse adiposity through suppressing lipogenesis in WAT; in particular, we observed a marked suppression of lipogenic genes LPL, FAS, and ACC1 in gonadal WAT by pregnancy in paroxetine-treated mice (Fig. 3). This attenuation in lipogenesis is apparently driven by increased estrogen production, given the well established effects of estrogen in suppressing lipogenic genes in WAT (D'Eon et al., 2005; Foryst-Ludwig and Kintscher, 2010). Consistent with this, we

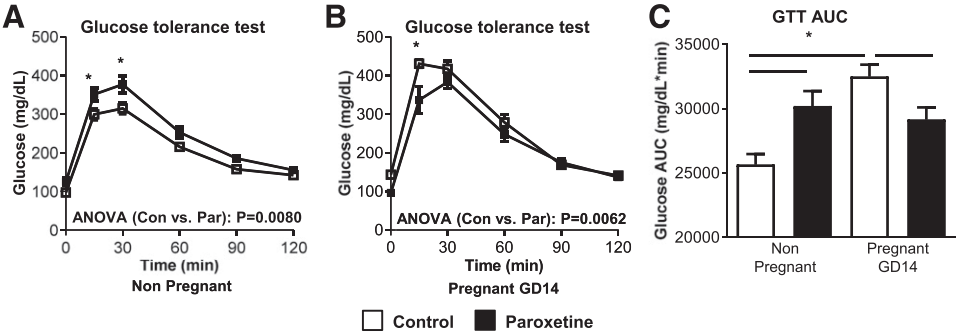

Fig. 4. Effect of paroxetine on glucose tolerance in non-pregnant and pregnant mice. (A and B) Glucose tolerance test (GTT) (16 hours of fasting) was performed on virgin and pregnant (GD14) WT mice after 12-week treatment with or without paroxetine (10 mg/kg per day) (n = 5–10 per group). The repeated area under the curve (AUC) measures ANOVA; P value is provided. The corresponding GTT AUC (C) was calculated. $^*P < 0.05$. Values are the mean ± S.E.M.

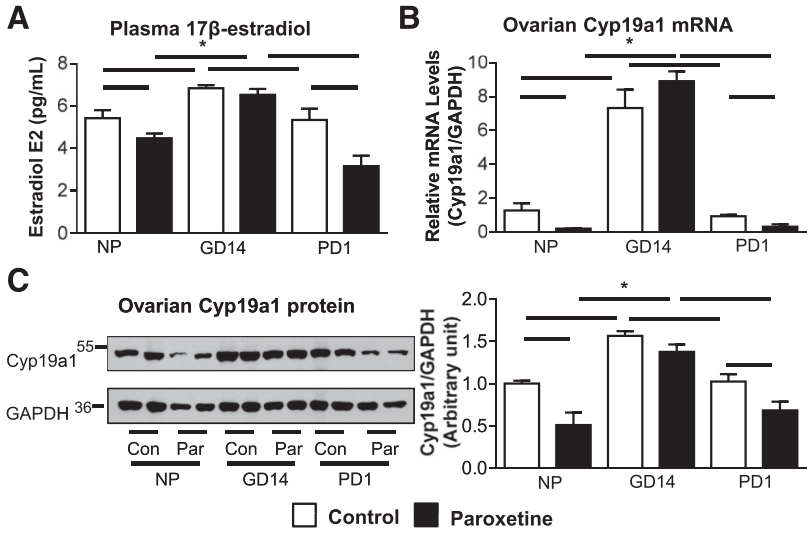

Fig. 5. Effect of paroxetine treatment on circulating 17β-E levels and ovarian CYP19a1 expression. (A) Plasma 17β-estradiol (17β-E) levels (n = 5 per group), (B) ovarian Cyp19a1 mRNA (n = 5 per group), and (C) protein levels (n = 3 per group) were measured in nonpregnant, pregnant (GD14), and PD 1 WT mice after 12-week treatment with or without paroxetine (10 mg/kg per day). *$P < 0.05$. Values are the mean ± S.E.M.

observed that increased lipogenesis in WAT and reduced circulating 17β-E levels co-occurred after delivery, as well as reduced lipogenesis in WAT from estrogen-treated mice. In addition, our experiments demonstrated that paroxetine treatment leads to abnormal lipid accumulation in BAT, but pregnancy appears to reverse these effects with upregulation of UCP1 (Fig. 3). BAT plays a pivotal role in thermogenesis and the regulation of energy expenditure, which is achieved by induction of UCP1 (Matthias et al., 2000). Upregulation of UCP1 has been previously shown to protect from obesity and associated insulin resistance (Kopecky et al., 1995), whereas our findings suggest that UCP1 expression is increased with pregnancy and is associated with prevention of adiposity by pregnancy. BAT thermogenesis stimulation is highly dependent on the sympathetic nervous system (SNS), and BAT function can be regulated by multiple mechanisms (Labbé et al., 2015). Norepinephrine released by the SNS efferent postganglionic fibers binds to the G-protein–coupled receptors, especially the β3-adrenergic receptor (ADRβ3) expressed in the brown adipocytes, which leads to a cascade of metabolic

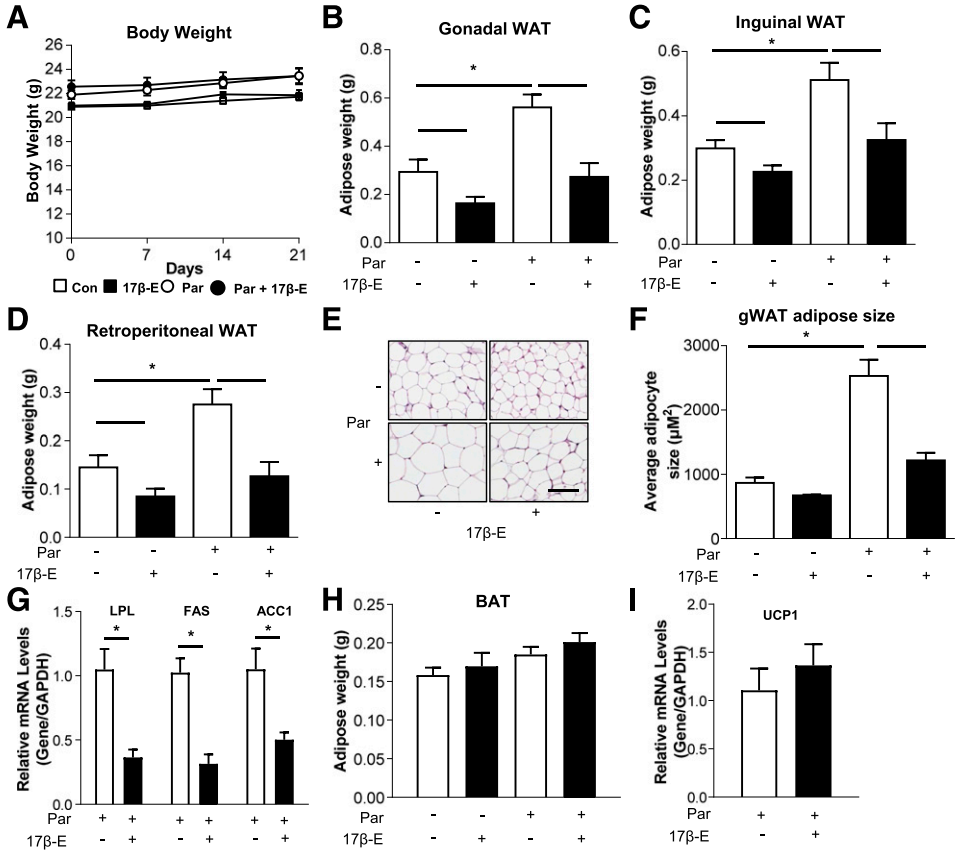

Fig. 6. Estrogen replacement reverses paroxetine-induced adiposity. WT mice were treated with paroxetine (10 mg/kg per day) in drinking water for 12 weeks. After 12 weeks, paroxetine treated- or -untreated mice were further treated with 17β-E (2 μg/day) or placebo control via subcutaneous implantation for 3 weeks. (A) Body weight changes for 15 weeks (n = 5 per group). (B–D and H) White and brown fat tissue weight at time of sacrifice (n = 5 per group). (E) Representative images of H&E-stained gWAT sections (scale bar, 100 μm). (F) Average adipocyte size per 10× field quantified using NIH ImageJ as described in *Materials and Methods*. Gene expression analysis in gWAT (G) and BAT (I) by quantitative reverse-transcribed polymerase chain reaction (n = 5 per group). *$P < 0.05$. Values are the mean ± S.E.

events triggering unimpeded substrate oxidation and heat production (Bachman et al., 2002; Lowell and Bachman, 2003). Previous studies have demonstrated that 17β-E activated BAT thermogenesis through the SNS by inhibiting hypothalamic AMP-activated protein kinase (Martinez de Morentin et al., 2014). 17β-E also regulates BAT directly by affecting ADRβ3 expression through epigenetic modifications (Al-Qahtani et al., 2017).

We observed that BAT mass and UCP-1 expression were not significantly increased by 17β-E replacement in the paroxetine-treated mice (Fig. 6, H and I), which is different from the effect of pregnancy on BAT in the paroxetine-treated mice (Figs. 2 and 3), possibly because the level of 17β-E achieved in the estrogen replacement study is not comparable to that gained during normal pregnancy. Regrettably, we did not monitor the serum level of 17β-E during the estrogen replacement experiment, which is a limitation of our study. Although previous studies have demonstrated that 17β-E can activate BAT thermogenesis through both central and direct signaling pathways (Schulz and Tseng, 2013), these studies either directly delivered estrogen to the central nervous system or dosed estrogen for a longer period. It is also possible that other pregnancy-related hormones play a coregulatory role in BAT function stimulation. Further investigation is needed to clarify the mechanisms underlying these differences.

The effects of pregnancy on paroxetine-induce CYP19a1 and estrogen suppression were reversible and ended soon after the litters were born. As early as PD 1, ovarian CYP19a1 expression and circulating 17β-E levels returned to prepregnancy state with the paroxetine-treated mice showing significantly lower CYP19a1 and 17β-E levels than the untreated group (Fig. 5). Accompanied by a reduction in estrogen levels, the expression of lipogenic genes (LPL, FAS, and ACC1) was already increased at PD 1 (Fig. 3). At PD 1, WAT mass and adipocyte size did not show a reversal as it may take longer to manifest the outcome of increased lipogenesis (Fig. 2). The reversal to decrease CYP19a1 expression, estrogen suppression, and increased expression of lipogenic genes in the paroxetine-treated mice after pregnancy also provides additional evidence to support estrogen suppression as the major mechanism underlying SSRI-induced obesity and metabolic defect in nonpregnant mice.

How paroxetine suppresses CYP19a1 expression in nonpregnant mice and how this suppression is removed by pregnancy but restored after birth are currently unclear. In most mammals, the production of estrogen under normal physiologic state is under the control of the hypothalamic-pituitary-gonadal (HPG) axis through the coordinated action of gonadotropin-releasing hormone and gonadotropins. By blocking SERT-mediated uptake, paroxetine elevates extracellular -hydroxytryptamine (serotonin) (5-HT) levels in the brain. It is possible that the heightened 5-HT tone may interact with the HPG axis to regulate CYP19a1 expression and estrogen production negatively. During pregnancy, the HPG axis is suppressed, and estrogen synthesis is modulated mainly by placental lactogens (Stocco, 2008), which apparently is not affected by SERT inhibition. Thus, the metabolic side effects of paroxetine are manifested only in the nonpregnant state.

In summary, our findings revealed a profound effect of pregnancy on normalizing SSRI-induced metabolic abnormalities and further demonstrated estrogen suppression as a major mechanism underlying the metabolic adverse effects associated with long-term use of SSRIs. Future studies are needed to delineate the molecular pathways underlying 5-HT and SSRI regulation of the HPG axis and the female endocrine system.

Acknowledgments

We thank the University of Washington Nutrition Obesity Research Center (supported by P30 DK035816), Pathology Histology Laboratory, and Pathology Digital Imaging Core Facility.

Authorship Contributions

Participated in research design: Zha, Hebert, Wang.
Conducted experiments: Zha, Hu.
Performed data analysis: Zha, Wang.
Wrote or contributed to the writing of the manuscript: Zha, Wang.

References

Al-Qahtani SM, Bryzgalova G, Valladolid-Acebes I, Korach-André M, Dahlman-Wright K, Efendić S, Berggren PO, and Portwood N (2017) 17β-Estradiol suppresses visceral adipogenesis and activates brown adipose tissue-specific gene expression. *Horm Mol Biol Clin Investig* **29**:13–26.
Alwan S and Friedman JM (2009) Safety of selective serotonin reuptake inhibitors in pregnancy. *CNS Drugs* **23**:493–509.
Andersohn F, Schade R, Suissa S, and Garbe E (2009) Long-term use of antidepressants for depressive disorders and the risk of diabetes mellitus. *Am J Psychiatry* **166**:591–598.
Andrade SE, Raebel MA, Brown J, Lane K, Livingston J, Boudreau D, Rolnick SJ, Roblin D, Smith DH, Willy ME, et al. (2008) Use of antidepressant medications during pregnancy: a multisite study. *Am J Obstet Gynecol* **198**:194.e1-e5.
Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, and Lowell BB (2002) betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* **297**:843–845.
Barbour LA (2014) Changing perspectives in pre-existing diabetes and obesity in pregnancy: maternal and infant short- and long-term outcomes. *Curr Opin Endocrinol Diabetes Obes* **21**:257–263.
Bérard A, Iessa N, Chaabane S, Muanda FT, Boukhris T, and Zhao JP (2016) The risk of major cardiac malformations associated with paroxetine use during the first trimester of pregnancy: a systematic review and meta-analysis. *Br J Clin Pharmacol* **81**:589–604.
Bérard A, Sheehy O, Damase-Michel C, and Crespin S (2012) Paroxetine use during pregnancy and perinatal outcomes including types of cardiac malformations in Quebec and France: a short communication. *Curr Drug Saf* **7**:207–210.
Chen X, Margolis KJ, Gershon MD, Schwartz GJ, and Sze JY (2012) Reduced serotonin reuptake transporter (SERT) function causes insulin resistance and hepatic steatosis independent of food intake. *PLoS One* **7**:e32511, 1–13.
Christensen H, Kupiec T, Jacobson J, Stewart J, Gonzalez C, and Rayburn W (1998) Tissue concentrations from consumption of paroxetine (paxil) in mice. *Neurotoxicol Teratol* **20**:365.
Cooper WO, Willy ME, Pont SJ, and Ray WA (2007) Increasing use of antidepressants in pregnancy. *Am J Obstet Gynecol* **196**:544.e1.
D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, and Greenberg AS (2005) Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem* **280**: 35983–35991.
Derijks HJ, Meyboom RH, Heerdink ER, De Koning FH, Janknegt R, Lindquist M, and Egberts AC (2008) The association between antidepressant use and disturbances in glucose homeostasis: evidence from spontaneous reports. *Eur J Clin Pharmacol* **64**:531–538.
Duan H and Wang J (2010) Selective transport of monoamine neurotransmitters by human plasma membrane monoamine transporter and organic cation transporter 3. *J Pharmacol Exp Ther* **335**:743–753.
Fava M, Judge R, Hoog SL, Nilsson ME, and Koke SC (2000) Fluoxetine versus sertraline and paroxetine in major depressive disorder: changes in weight with long-term treatment. *J Clin Psychiatry* **61**:863–867.
Foryst-Ludwig A and Kintscher U (2010) Metabolic impact of estrogen signalling through ERalpha and ERbeta. *J Steroid Biochem Mol Biol* **122**:74–81.
Hennings JM, Schaaf L, and Fulda S (2012) Glucose metabolism and antidepressant medication. *Curr Pharm Des* **18**:5900–5919.
Hiemke C and Härtter S (2000) Pharmacokinetics of selective serotonin reuptake inhibitors. *Pharmacol Ther* **85**:11–28.
Homberg JR, la Fleur SE, and Cuppen E (2010) Serotonin transporter deficiency increases abdominal fat in female, but not male rats. *Obesity (Silver Spring)* **18**: 137–145.
Kahn BB and Flier JS (2000) Obesity and insulin resistance. *J Clin Invest* **106**: 473–481.
Kopecky J, Clarke G, Enerbäck S, Spiegelman B, and Kozak LP (1995) Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J Clin Invest* **96**:2914–2923.
Kulkarni J, Storch A, Baraniuk A, Gilbert H, Gavrilidis E, and Worsley R (2015) Antipsychotic use in pregnancy. *Expert Opin Pharmacother* **16**:1335–1345.

Labbé SM, Caron A, Lanfray D, Monge-Rofarello B, Bartness TJ, and Richard D (2015) Hypothalamic control of brown adipose tissue thermogenesis. *Front Syst Neurosci* **9**:150, 1–13.
Lain KY and Catalano PM (2007) Metabolic changes in pregnancy. *Clin Obstet Gynecol* **50**:938–948.
Lee N, Hebert MF, Prasad B, Easterling TR, Kelly EJ, Unadkat JD, and Wang J (2013) Effect of gestational age on mRNA and protein expression of polyspecific organic cation transporters during pregnancy. *Drug Metab Dispos* **41**: 2225–2232.
Lindqvist PG, Nasiell J, Gustafsson LL, and Nordstrom L (2014) Selective serotonin reuptake inhibitor use during pregnancy increases the risk of postpartum hemorrhage and anemia: a hospital-based cohort study. *J Thromb Haemost* **12**: 1986–1992.
Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods* **25**: 402–408.
Lowell BB and Bachman ES (2003) Beta-adrenergic receptors, diet-induced thermogenesis, and obesity. *J Biol Chem* **278**:29385–29388.
Marcus SM, Flynn HA, Blow FC, and Barry KL (2003) Depressive symptoms among pregnant women screened in obstetrics settings. *J Womens Health (Larchmt)* **12**: 373–380.
Martínez de Morentin PB, González-García I, Martins L, Lage R, Fernández-Mallo D, Martínez-Sánchez N, Ruíz-Pino F, Liu J, Morgan DA, Pinilla L, et al. (2014) Estradiol regulates brown adipose tissue thermogenesis via hypothalamic AMPK. *Cell Metab* **20**:41–53.
Matthias A, Ohlson KB, Fredriksson JM, Jacobsson A, Nedergaard J, and Cannon B (2000) Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty scid-induced thermogenesis. *J Biol Chem* **275**:25073–25081.
McGlashon JM, Gorecki MC, Kozlowski AE, Thirnbeck CK, Markan KR, Leslie KL, Kotas ME, Potthoff MJ, Richerson GB, and Gillum MP (2015) Central serotonergic neurons activate and recruit thermogenic brown and beige fat and regulate glucose and lipid homeostasis. *Cell Metab* **21**:692–705.
McIntyre RS, Soczynska JK, Konarski JZ, and Kennedy SH (2006) The effect of antidepressants on glucose homeostasis and insulin sensitivity: synthesis and mechanisms. *Expert Opin Drug Saf* **5**:157–168.
Pedroni SM, Turban S, Kipari T, Dunbar DR, McInnes K, Saunders PT, Morton NM, and Norman JE (2014) Pregnancy in obese mice protects selectively against visceral adiposity and is associated with increased adipocyte estrogen signalling. *PLoS One* **9**:e94680, 1–11.
Raeder MB, Bjelland I, Emil Vollset S, and Steen VM (2006) Obesity, dyslipidemia, and diabetes with selective serotonin reuptake inhibitors: the Hordaland Health Study. *J Clin Psychiatry* **67**:1974–1982.
Riant E, Waget A, Cogo H, Arnal JF, Burcelin R, and Gourdy P (2009) Estrogens protect against high-fat diet-induced insulin resistance and glucose intolerance in mice. *Endocrinology* **150**:2109–2117.
Schulz TJ and Tseng YH (2013) Systemic control of brown fat thermogenesis: integration of peripheral and central signals. *Ann N Y Acad Sci* **1302**:35–41.
Serretti A and Mandelli L (2010) Antidepressants and body weight: a comprehensive review and meta-analysis. *J Clin Psychiatry* **71**:1259–1272.
Steiner M (1998) Perinatal mood disorders: position paper. *Psychopharmacol Bull* **34**: 301–306.
Stocco C (2008) Aromatase expression in the ovary: hormonal and molecular regulation. *Steroids* **73**:473–487.
Stubbins RE, Holcomb VB, Hong J, and Núñez NP (2012) Estrogen modulates abdominal adiposity and protects female mice from obesity and impaired glucose tolerance. *Eur J Nutr* **51**:861–870.
Tang SW and Helmeste D (2008) Paroxetine. *Expert Opin Pharmacother* **9**:787–794.
Toh S, Mitchell AA, Louik C, Werler MM, Chambers CD, and Hernández-Díaz S (2009) Selective serotonin reuptake inhibitor use and risk of gestational hypertension. *Am J Psychiatry* **166**:320–328.
Wu J, Cohen P, and Spiegelman BM (2013) Adaptive thermogenesis in adipocytes: is beige the new brown? *Genes Dev* **27**:234–250.
Zha W, Edin ML, Vendrov KC, Schuck RN, Lih FB, Jat JL, Bradbury JA, DeGraff LM, Hua K, Tomer KB, et al. (2014) Functional characterization of cytochrome P450-derived epoxyeicosatrienoic acids in adipogenesis and obesity. *J Lipid Res* **55**: 2124–2136.
Zha W, Ho HTB, Hu T, Hebert MF, and Wang J (2017) Serotonin transporter deficiency drives estrogen-dependent obesity and glucose intolerance. *Sci Rep* **7**:1137, 1–14.

Address correspondence to: Dr. Joanne Wang, Department of Pharmaceutics, University of Washington, H272J, Health Sciences Bldg., Seattle, WA 98195-7610. E-mail: jowang@u.washington.edu