HHS Public Access

Author manuscript

Int J Obes (Lond). Author manuscript; available in PMC 2019 September 05.

Published in final edited form as:

Int J Obes (Lond). 2019 September ; 43(9): 1747–1758. doi:10.1038/s41366-018-0304-x.

High-fat Feeding Reprograms Maternal Energy Metabolism and Induces Long-term Postpartum Obesity in Mice

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Abstract

Background—Excessive gestational weight gain (EGWG) closely associates with postpartum obesity. However, the causal role of EGWG in postpartum obesity has not been experimentally verified. The objective of this study was to determine whether and how EGWG causes long-term postpartum obesity.

Methods—C57BL/6 mice were fed with high-fat diet during gestation (HFFDG) or control chow, then their body composition and energy metabolism were monitored after delivery.

Results—We found that HFFDG significantly increased gestational weight gain. After delivery, adiposity of HFFDG-treated mice (Preg-HF) quickly recovered to the levels of controls. However, three months after parturition, Preg-HF mice started to gain significantly more body fat even with regular chow. The increase of body fat of Preg-HF mice was progressive with aging and by 9 months after delivery had increased 2-fold above the levels of controls. The expansion of white adipose tissue (WAT) of Preg-HF mice was manifested by hyperplasia in visceral fat and hypertrophy in subcutaneous fat. Preg-HF mice developed low energy expenditure and UCP1 expression in interscapular brown adipose tissue (iBAT) in later life. Although blood estrogen concentrations were similar between Preg-HF and control mice, a significant decrease in estrogen receptor α (ERα) expression and hypermethylation of the ERα promoter was detected in the fat of Preg-HF mice nine months after delivery. Interestingly, hypermethylation of ERα promoter and low ERα expression were only detected in adipocyte progenitor cells in both iBAT and WAT of Preg-HF mice at the end of gestation.

Conclusions—These results demonstrate that HFFDG causes long-term postpartum obesity independent of early postpartum fat retention. This study also suggests that HFFDG adversely

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Author Contributions

L.Q., K.C., J.S.W., S.L. H.G. and G.S.F. contributed research data. L.Q., W.W.H.Jr. and J.S. contributed to research design and manuscript preparation.

Conflict of Interest

All authors have no conflict of interest to declare.

programs long-term postpartum energy metabolism by epigenetically reducing estrogen signaling in both BAT and WAT.

Keywords

Pregnancy; obesity; gestational weight gain; brown adipose tissue

Introduction

Since 2014, women have overtaken men in obesity by 4% in U.S. [1, 2]. CDC studies also have found that there was a significant increase in obesity among women aged 60 and older [1, 3]. Gestation is a unique physiological process that most women go through during child-bearing age. To ensure fetal nutrient supply and lactation, maternal metabolism goes through a series of adaptations. Among these adaptations, gaining maternal body fat during pregnancy is predominant [4]. Gaining proper weight during pregnancy has immediate and long-term health implications for both offspring and mothers. Compelling clinical data indicate that excessive gestational weight gain (EGWG) independently predicts long-term (up to decades) postpartum maternal obesity [5–10]. However, little information is available regarding whether and how EGWG causes long-term postpartum maternal obesity. Conflicting results from studies of postpartum weight retention even have led to the notion that long-term postpartum obesity is a continuation of pregnancy-associated weight gain or fat retention [5–12].

Although mice have a relative short pregnancy, gestation robustly increases maternal body fat mass [4]. These results led us to use mice as an animal model to study the causal relationship between EGWG and long-term postpartum obesity. We fed pregnant C57BL/6 mice with a high-fat (HF) diet and monitored maternal adiposity and energy metabolism. Our studies showed that HF feeding during gestation (HFFDG) of mice resembles most metabolic phenotypes of EGWG-associated long-term postpartum obesity in humans. Most importantly, the quick recovery of adiposity after delivery indicates that HFFDG-induced long-term postpartum obesity is independent of postpartum fat retention. Therefore, the current study indicates that HFFDG adversely reprograms postpartum maternal energy metabolism and lead to a higher incidence of obesity.

Material and Methods

Materials.

Glucose, insulin, T3, collagenase and Dulbecco’s modified Eagle’s medium (DMEM) were from Sigma-Aldrich (St. Louis, MO). Antibody against estrogen receptor α (ERα) and PE-conjugated anti-Sca-1 or PDGFRα antibodies were from R&D System (Minneapolis, MN). The anti-UCP1 antibody, FITC-conjugated antibody against CD29 or CD34, and mouse estradiol ELISA kit were purchased from the Abcam (Cambridge, MA). Anti-GAPDH antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). NuPAGE gels, SuperScript III reverse transcriptase and oligo$(dT)_{12–18}$ primers were from Invitrogen (Carlsbad, CA). The mouse diabetes multiplex assay kit was from Bio-Rad (Hercules, CA). HF diet (60 kCal% from fat, 20 kCal% from protein, 20 kCal% from carbohydrate, energy

density: 5.24 kCal/g, catalog number D12492) was from Research Diets, Inc (New Brunswick, NJ). Regular chow (17 kCal% from fat, 25 kCal% from protein, 58 kCal% from carbohydrate, energy density: 3.1 kCal/g, catalog number 7912) was from Harlan Laboratories (Madison, WI).

Experimental animals.

C57BL/6 mice were from the Jackson Laboratory (Bar Harbor, ME). Ten to 12-week-old nulliparous female mice were randomly selected for mating. Normal C57BL6 sires or vasectomized male mice were fed with regular chow. Pregnancy was determined by the presence of a vaginal plug and was assigned the embryonic day (E) 0.5. Female mice mated with vasectomized males were used as non-pregnant controls. The pregnant or non-pregnant mice were randomly assigned to feed with HF or chow. One group of non-pregnant mice were fed with HF diet for 20 days. Therefore, there were 4 groups of mice: 1) non-pregnant chow-fed (Non-Preg-C); 2) non-pregnant HF-fed (Non-Preg-HF); 3) pregnant and fed with chow (Preg) and 4) pregnant and fed with HF (Preg-HF). After delivery, all mice were fed with chow. Body composition was determined by Echo-MRI (Houston, TX). Glucose tolerance tests were performed 9 months after delivery after 6-hour fasting with i.p. injection of glucose (2 g/kg of body weight). Tissues samples were collected in the fed state at E18.5 and 36-week after delivery. Experiments using mouse models were carried out under the Association for Assessment and Accreditation of Laboratory Animal Care guidelines with approval from the University of California San Diego Animal Care and Use Committee.

Fat morphology study:

Adipocyte areas of WAT were measured using ImageJ software (NIH, Bethesda, MA), with at least 9 images of the randomly chosen area of one H&E stained section [13]. Adipocytes were outlined to measure the cross-section areas, excluding those that were too small or too large (<100 or >4,800 arbitrary units).

Precursor cell-derived adipocyte differentiation.

The precursor cells were isolated by fluorescence-activated cell sorting (FACS) of the stromal vascular fraction (SVF) of mouse iBAT or gonadal WAT (gWAT) [14, 15]. Briefly, SVFs were resuspended in PBS and incubated with anti-CD34, Sca-1 and PDGFRα antibodies (iBAT) or anti-CD34, Sca-1 and CD29 antibodies (gWAT) for 30 min on ice. Sorted cells were proliferated in DMEM supplemented with 20% FBS and 2.5 ng/ml bFGF for 2 weeks. Precursor cells from iBAT were cultured in a differentiation medium (DMEM with 10% FBS, 20 nmol/l insulin and 1 nmol/l T3) for 3 days and then switched to induction medium (differentiation medium plus 0.125 mmol/l indometacin, 5 μmol/l dexamethasone and 0.5 mmol/l IBMX). Two days later, the cells were switched back to differentiation medium for 5 days [14]. Differentiated cells were stained with oil red O. Precursor cells from gWAT were induced to differentiate into white adipocytes as previously described [16].

Indirect Calorimetry, Western blot, and real-time PCR assays.

Used the same procedures as we previously described [4, 13]. Sequence of specific primers are in Table 1.

Measurement of methylation of the ERα promoter.

The methylation status of specific CpG dinucleotides within the exon A and C of mouse ERα promoter was determined by sodium bisulfite modification and pyrosequencing [17]. Genomic DNA sample (1 μg) was modified by sodium bisulfate with an EpiTect Bisulfite kit (Qiagen). Modified DNA was used as a template and DNA fractions crossing the CpG sites of mouse ERα promoter were amplified by PCR using two pairs of primers: exon A, forward 5'-TGGGTTATTTGTGTTTTGTAGGATAG-3" and reverse 5'-CTTAAATCTAATACAACAAAACCATTC-3'; exon C, forward 5'-TGTTAAGTGTTTTGTTTATTGGTTG-3' and reverse 5'-CCTCTTTCCAAAAATATTCCATAAATT-3' [17]. The methylation level at the CpG sites of ERα promoter was determined and quantified by pyrosequencing and PyroMark Q24 software (Qiagen).

Statistical analysis.

Data are expressed as a mean ± standard error of the mean (SEM). Statistical analyses were performed using the Student t-test or two-way ANOVA, followed by Bonferroni post-tests using Prism software. Differences were considered significant at $p<0.05$.

Results

HFFGD induced EGWG and long-term postpartum maternal obesity.

Mice are altricial, and WAT is developed postnatally. Therefore, the measurement of body fat by Echo-MRI in a pregnant mouse represents essentially only maternal adiposity. During gestation, there were significant increases in body fat and body weight of Preg mice (Fig. 1a&b). Comparing Preg-HF with Preg dams, there was significantly more body fat and body weight gained in Preg-HF mice from early to the end of gestation (Fig. 1a&b). Litter sizes were comparable among the groups of mice (data not shown). Interestingly, a large decrease in body fat during delivery was observed in both Preg and Preg-HF dams (Fig. 1a).

To focus on the effect of EGWG during pregnancy and avoid effects of lactation on maternal metabolism, regular chow was provided to all mice immediately after delivery and pups were removed to stop lactation. Soon after delivery, body weight and fat content of Preg-HF mice was restored to the levels of Preg and Non-Preg-C mice (Fig. 1a&b). However, three months later, Preg-HF mice started to gain significantly more body fat than the other three groups of control mice, and the differences were more pronounced with aging (Fig. 1a). Nine months after delivery, body fat content of Preg-HF mice was 2-times higher than all control mice (Fig. 1a&c). In addition, impaired glucose tolerance (Fig. 1d), and elevated blood FFA and TG concentrations (Supplemental Fig. 1a&b) were detected in Preg-HF mice 9 months after delivery. Together, these results indicate that HFFDG induces not only EGWG but also long-term postpartum obesity and metabolic defects.

As expected, HF feeding of age-matched non-pregnant mice significantly increased their body fat (Fig. 1a). After switching back to regular chow, Non-Preg-HF mice gained similar amount of body fat as Non-Preg-C and Preg mice (Fig. 1a), indicating that brief HF feeding at pup-bearing age does not induce obesity in later life. In addition, there were no

differences in postpartum body fat content (Fig. 1a), body weight (Fig. 1b), and insulin sensitivity (Fig. 1d) between Preg and Non-Preg-C mice, indicating that single normal pregnancy itself does not alter postpartum maternal metabolism.

HFFDG induced long-term postpartum hyperplasia and hypertrophy of WAT in a depot-specific manner.

Expansion of WAT mass is caused by increasing adipocyte number (hyperplasia) and/or adipocyte volume (hypertrophy). We analyzed the WATs from Preg-HF and control mice 9 months after delivery. The average adipocyte area of gonadal WAT (gWAT) from Preg-HF mice was significantly smaller than that of Preg mice (Fig. 2a). Comparison of the frequency distribution of adipocyte area revealed that there were significantly more small adipocytes in gWAT of Preg-HF mice (Fig. 2b). In contrast, average adipocyte area of inguinal WAT (iWAT) of Preg-HF was significantly greater than that of other three groups (Fig. 2c), while high frequencies of remarkably large adipocytes were found in iWAT of Preg-HF mice (Fig. 2d). These data indicate that the increase of visceral WAT of Preg-HF mice in later postpartum life is mainly due to small adipocytes, while the expansion of subcutaneous WAT is mainly caused by hypertrophy.

Increased body fat was observed in Preg-HF mice during pregnancy and in late life (Fig. 1a). To study if the postpartum obese phenotype of Preg-HF mice is an extension of HFFDG-induced adiposity, we analyzed the WAT samples from dams at E18.5. Interestingly, average adipocyte areas of gWAT from Preg-HF and Non-Preg-HF were similarly increased compared with that of Non-Preg-C or Preg mice (Fig. 2e). Inguinal WAT was not analyzed because of the mix of the mammary gland during pregnancy. There were higher frequencies of large adipocytes in gWAT of Preg-HF and Non-Preg-HF mice at E18.5 (Fig. 2f). These results indicate that similar to Non-Preg-HF mice, HF feeding of pregnant mice increased body fat mainly via hypertrophy.

Most importantly, when comparing the differences in adipocyte area and frequency distribution of gWAT between Preg-HF and Preg mice at E18.5 (Fig. 2e) and 9-months after delivery (Fig. 2a), there was a marked transition from enlarged adipocytes to a larger number of small adipocytes in visceral WAT of Preg-HF mice during the development of obesity in later life. These studies further support the concept that EGWG programs long-term postpartum maternal metabolic defects and obesity rather than retention of excess fat gained during pregnancy.

HFFDG reduced long-term postpartum energy expenditure and thermogenesis.

Except for very rare cases of genetic mutations, obesity is primarily caused by chronic energy imbalance from a variety of environmental conditions. In this regard, measurements of whole-body energy metabolism of the mice 8 months after delivery showed a significantly lower energy expenditure rate in Preg-HF mice (Fig. 3a). However, food intake, locomotor activity (data not shown) and respiratory exchange ratio (RER) (Fig. 3b) were similar among the 4 groups of mice. Since non-shivering thermogenesis plays an important role in energy expenditure, we then analyzed iBAT and found that, although there was no change in tissue mass (Fig. 1c), Preg-HF mice had significantly fewer brown adipocytes but larger lipid

vacuoles (Fig. 3c&d). Our study also revealed that UCP1 expression levels at both protein (Fig. 3e) and mRNA levels (Fig. 3f) were significantly decreased in iBAT of Preg-HF mice. In addition, mRNA levels of Dio2, PPARα, PGC-1α, Cox5α, Zfb516, and Cidea, but not β3 adrenoreceptor (Adrb3), PRDM16, C/EBPβ, and PPARγ, were remarkably decreased in iBAT from Preg-HF mice (Fig. 3f). Most importantly, during acute cold exposure, core body temperatures of Preg-HF mice decreased more than those of the controls (Fig. 3g). Together, these results indicate that there were significant decreases in energy expenditure and BAT-derived thermogenesis in later life of Preg-HF mice.

HF feeding enhanced UCP1 expression and energy expenditure during pregnancy.

UCP1 expression and BAT activation are suppressed during gestation [18–21], which raise the question of whether HFFDG enhances the inhibitory effect of pregnancy on BAT thermogenesis, but then maintains this inhibitory effect in postpartum life. To address this question, we studied the effect of HFFDG on energy metabolism and BAT activation during pregnancy. Consistent with previous reports, we found a steady decrease of UCP1 expression in iBAT during pregnancy (data not shown) and reduction of energy expenditure in Preg mice (Fig. 4a&b), which further supports the concept that decreased BAT activity contributes to the adaptive decrease of maternal energy expenditure. Surprisingly, in contrast to our hypothesis, UCP1 expression (Fig. 4c) and energy expenditure rates of Preg-HF mice were remarkably higher than those of chow-fed Preg mice (Fig. 4b). Locomotor activities were similar between Preg-HF and Preg mice (Fig. 4d). However, energy intakes of Preg-HF mice were significantly higher than Preg mice (Fig. 4e). Together, these results indicate that, instead of reducing UCP1 expression and energy expenditure, HFFDG enhances BAT activation and energy expenditure during pregnancy. Of note, induction of UCP1 expression by HF feeding has been well documented in rodent models [22–25].

Comparing energy metabolism and UCP1 expression in iBAT from Preg-HF with those of Preg mice at E18.5 or 9 months after delivery, our studies revealed a reversed development of energy metabolism of Preg-HF mice, from increased UCP1 expression and energy expenditure during pregnancy to decreased UCP1 expression and energy expenditure in the late postpartum period. This change in energy metabolism parallels the reduction in brown adipocyte number and UCP1 expression in iBAT of Preg-HF mice. Similar to white adipocytes, brown adipocytes are postmitotic. Brown adipocyte renewal and hyperplastic growth of BAT are dependent on brown adipocyte progenitors. To study what causes the reduction of brown adipocyte number and a postpartum decrease of UCP1 expression in iBAT in Preg-HF mice, we isolated precursor cells from the stromal vascular fraction of iBAT at E18.5 and induced them to differentiate into brown adipocytes [26, 27]. As shown in figure 4f, fewer brown adipocytes were produced from iBAT of Preg-HF dams. These results suggest that an increase in UCP1 protein levels in iBAT of Preg-HF mice at E18.5 occurs through increased UCP1 expression in pre-existing brown adipocytes rather than recruitment of new adipocytes. Decreased brown precursor cell differentiation potency most likely contributes to decreased postpartum brown adipocyte number.

HFFDG reduced estrogen singling in postpartum fat.

Estrogen plays an important role in energy metabolism through a variety of mechanisms including food intake and direct regulation of lipid metabolism in peripheral tissues [28–30]. Estrogen deficiency, which occurs post-menopausal, induces fat accumulation and obesity [31]. Using ovariectomized mice and estradiol reconstitution, our study confirmed that estrogen deficiency increases fat deposition and reduces UCP1 expression in iBAT in mice (Supplemental Fig. 2).

The main characteristic of estrogen deficiency-induced obesity is an expansion of visceral fat [28–30]. Our preliminary studies showed that the increase of gWAT of Preg-HF mice had a trend to be greater than other WATs (Fig. 1c), which led us to speculate that estrogen is involved in the development of postpartum obesity of Preg-HF mice. However, there were no significant changes in food intake and activity in Preg-HF mice, which most likely exclude the involvement of the estrogen/CNS pathway. Our results also suggest that, if estrogen is involved, it is unlikely through a systemic estrogen deficiency but rather through its local effects, because blood estradiol concentrations were similar between Preg-HF and controls (data not shown).

There are 2 estrogen receptors: ERα and ERβ. The metabolic effects of estrogen are primarily mediated by ERα [28, 29, 32]. We measured the protein levels of ERα in metabolically active tissues 9 months after delivery. Expression levels of ERα protein and mRNA were significantly reduced in both iBAT and WAT in Preg-HF mice (Fig. 3e, 5a&b). However, there were no changes of ERα in the liver, skeletal muscle, or hypothalamus (Fig. 5b). Accordingly, mRNA levels of estrogen-responsive gene EBAG9 (estrogen receptor-binding fragment-associated antigen 9)[33] were significantly decreased in fat but showed no change in liver, skeletal muscle, or hypothalamus of Preg-HF (Supplemental Figure 3). These results suggest that estrogen/ERα signaling was impaired in adipose tissues of Preg-HF mice.

Maternal HF feeding increased methylation of the ERα promoters in adipocyte progenitors.

Reduction of ERα mRNA in WAT and BAT of Preg-HF mice suggests that the inhibition of ERα expression is likely at the transcriptional level. Methylation of ERα promoter suppresses ERα expression [17, 34–38]. For the fat samples collected 9-months after delivery, the methylation levels of exons A and C within the mouse ERα promoter [39] were significantly increased in iWAT, gWAT, and iBAT (Fig. 5c). However, for the fat samples collected at E18.5, similar to ERα protein levels, there was no significant change in DNA methylation of the ERα promoters of Preg-HF mice (data not shown). These results indicate that increased methylation of the ERα promoters may mediate HFFDG-programmed lower ERα expression in adipose tissue in later life. These results also raise a question about how HFFDG programs such long-term effects on ERα gene expression in fat.

DNA methylation is stable and heritable during mitotic division and even differentiation processes. There is a renewal process in adipose tissue, through which progenitor-derived new adipocytes replace older mature adipocytes. Therefore, if adipocyte precursor cells were epigenetically programmed by HFFDG, increased DNA methylation at the ERα promoters

of the progenitor cells could be passed to the renewed mature adipocytes. Therefore, we isolated adipocyte progenitors from the dams' gWAT ($CD29^+$:$CD34^+$:$Sca\text{-}1^+$) and iBAT ($PDGFR\alpha^+$:$CD34^+$:$Sca\text{-}1^+$) at E18.5 using FACS [40, 41]. We found that the methylation levels at the ERα promoters were significantly increased in both white and brown APs from Preg-HF mice (Fig.5d). Furthermore, after *in vitro* differentiation using sorted APs, hypermethylation at the ERα promoter regions was maintained (data not shown).

Discussion

To the best of our knowledge, this is the first experimental observation showing that HFFDG adversely programs lower rates of postpartum maternal energy expenditure and greater obesity, both independent of postpartum weight retention. This study also demonstrates that pregnancy opens a vulnerable window to resetting of maternal energy metabolism. As with fetal programming, which refers to the life-long effects of maternal nutrition on offspring' health, we identify the process of HFFDG-induced long-term postpartum obesity and metabolic defects as maternal programming.

EGWG increases the risk of long-term postpartum obesity. However, it still is debatable whether EGWG causes the development of postpartum metabolic defects and obesity, especially when EGWG is complicated with postpartum fat retention. Using a HFFDG approach, our study revealed that excessive maternal energy intake significantly increased WAT weight gain, but this did not occur in other tissues (Supplemental Figure 4), which is a key characteristic in human EGWG [42]. Similar to humans [5–10], female mice who received HFFDG spontaneously developed obesity several months after delivery. Most importantly, our study demonstrates that soon after delivery, adiposity of Preg-HF mice recovers to the same levels as controls. Therefore, these data demonstrate that HFFDG-induced postpartum obesity is not an extension of pregnancy-induced fat gain and postpartum fat retention in mice. We recognize that mouse gestation is relatively short and equals to the first and second trimester of human pregnancy. Therefore, HF feeding-induced EGWG in mice resembles the metabolic phenotypes in the early gestation in humans. However, women with EGWG during the first trimester have a much higher risk of developing long-term postpartum metabolic defects than mothers who experienced EGWG in the second and third trimester [8]. Therefore, despite the short gestation, HFFDG of the mouse provides a useful animal model to study EGWG-programmed long-term postpartum obesity. We also realize that the HF diet used in this study has 60% calories from fat, which is considered an unphysiological diet. The main reason for using this HF diet is to produce the obese phenotype in the short pregnancy period in female mice, who are more resistant than male mice to diet-induced obesity [43, 44]. More studies are required to determine whether other HF diets with lower fat content can reproduce similar EGWG and postpartum obesity phenotypes. In addition, the current study does not allow us to precisely distinguish the causal roles of caloric intake versus dietary fat (especially fatty acids) composition of the HF diet in HFFDG-induced postpartum obesity.

The high concurrence of pre-pregnant obesity and postpartum weight retention in women who experience EGWG complicates understanding about whether and how EGWG alters long-term postpartum energy metabolism. By controlling energy intake during gestation, our

current study allowed us to focus on the effect of HFFDG alone. Our results indicate that without pre-pregnant obesity and postpartum fat retention, HFFDG can program lower rates of maternal energy expenditure and induce late postpartum obesity. The steady increase of adiposity of Preg-HF mice without any diet challenge further reveals the large degree of HFFDG-impaired postpartum energy metabolism. Our study also does not support the notion that EGWG-associated long-term postpartum obesity is a continuation of pregnancy-induced fat expansion and retention. Of note, removal pups after birth prevented us from studying the role of lactation in HFFDG-programmed postpartum obesity. Eliminating other factors such as lactation further demonstrates the prominence and casual role of HFFDG in the development of postpartum metabolic defects in mice.

Accumulative energy imbalance plays an essential role in the development of obesity. Prolonged HF feeding alters eating behavior of female mice [45]. Perhaps because of the relative short period of HF feeding, our results revealed that HFFDG showed no significant effect on food intake or locomotor activity of mice during the long-term postpartum period. Decreased energy expenditure rates of Preg-HF mice suggest that energy imbalance underlies HFFDG-programmed postpartum obesity. The thermogenic activity of brown adipocytes is inversely correlated with BMI, which indicates that brown adipocytes play a key role in energy metabolism and obesity [46–49]. Our results showed that the expression levels of UCP1 and major mitochondrial markers were significantly reduced in iBAT of Preg-HF mice several months after delivery. In addition, these mice were cold-intolerant. Therefore, we postulate that decreased BAT thermogenesis might cause the reduction of energy expenditure in maternal programming. Interestingly, our study also showed that, in contrast to the inhibitory effect of gestation on thermogenesis, HFFDG robustly enhances UCP1 expression and energy expenditure in pregnant mice. Again, the conversion of HF-increased energy expenditure rate during gestation to low energy expenditure rate in later life indicates that EGWG programs a long process of reducing of BAT activity. The dynamic changes in WAT morphology also suggest that HFFDG-programmed WAT expansion is not a simple adipocyte size enlargement. Both white and brown adipocytes are postmitotic. Adipocyte renewal rate and functional characteristics of renewed adipocytes play key roles in maintaining WAT and BAT functions. The changes in tissue morphology and function of WAT and BAT in Preg-HF mice later life prompt us to propose that HFFDG might reprogram postpartum adipocyte renewal. Therefore, further studies are required to elucidate the role of adipocyte renewal and metabolism of both WAT and BAT in maternal programming.

Estrogen plays an important role in maintaining energy homeostasis and fat tissue development [50]. Our study revealed that WAT distribution, fat morphology, and UCP1 expression of HFFDG-induced long-term postpartum obese mice were similar to estrogen deficient patients and ovariectomized mice. However, our data indicate that HFFDG impairs ERα-mediated estrogen signaling in fat but not through a systemic reduction of hormone concentration and CNS. Systemic or tissue-specific (e.g., hypothalamus or adipocyte), ERα gene knockouts lead to obesity and insulin resistance in mice [32, 50–53]. In addition, estrogen inhibits the commitment of white adipocyte lineage and adipocyte differentiation of mesenchymal stem cells through ERα [54–57]. Our study demonstrated that HFFDG reduces ERα expression by increasing methylation of its promoter in adipocyte precursor cells.

Although the current study did not provide any evidence showing how HFFDG selectively increases ERα promoter methylation, epigenetic modification of ERα expression in adipocyte precursor cells provides an underlying mechanism through which HFFDG programs long-term postpartum obesity. We postulate that through adipocyte precursor cell-directed postpartum adipocyte renewal, hypermethylation of ERα promoter and low ERα expression gradually become dominant in adipose tissues at the later time point. Further studies are required to verify if hypermethylation of the ERα promoter and impaired estrogen signaling mediate HFFDG-programmed long-term postpartum metabolic defects and obesity.

In summary, by feeding C57BL/6 mice with a HF diet during pregnancy, our study demonstrates that HFFDG adversely programs long-term postpartum metabolic defects and obesity, but this does not occur through postpartum fat retention. This study also reveals that HFFDG-programmed long-term postpartum obesity may occur through adipocyte precursor cell-directed WAT adipogenesis and thermogenesis in BAT. Most importantly, our current study demonstrates that pregnancy opens a vulnerable window for resetting maternal metabolism. If HFFDG-programmed metabolic defects, i.e. maternal programming, can be verified in human subjects with EGWG, it will provide an explanation for how women have overtaken men in the obesity epidemic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH grants HD069634 (J.S.), DK095132 (J.S.), HD007186 (W.W.H.Jr), HD068372(W.W.H.Jr), UL1TR001082(W.W.H.Jr), and American Diabetes grant 1-16-IBS-272 (J.S.). Dr. Jianhua Shao is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Abbreviations:

EGWG	excessive gestational weight gain
HFFDG	high fat feeding during gestation
HF	high-fat
iBAT	interscapular brown adipose tissue
WAT	white adipose tissue
gWAT	gonadal WAT
iWAT	inguinal WAT
ERα	estrogen receptor α
Non-Preg-C	non-pregnant chow-fed mice

Non-Preg-HF	non-pregnant HF-fed mice
Preg	pregnant mice and fed with chow
Preg-HF	pregnant and fed with HF
UCP1	uncoupling protein 1
PPARγ	peroxisome proliferator–activated receptor γ
PGC-1α	PPARγ co-activator 1 α
RER	respiratory exchange ratio

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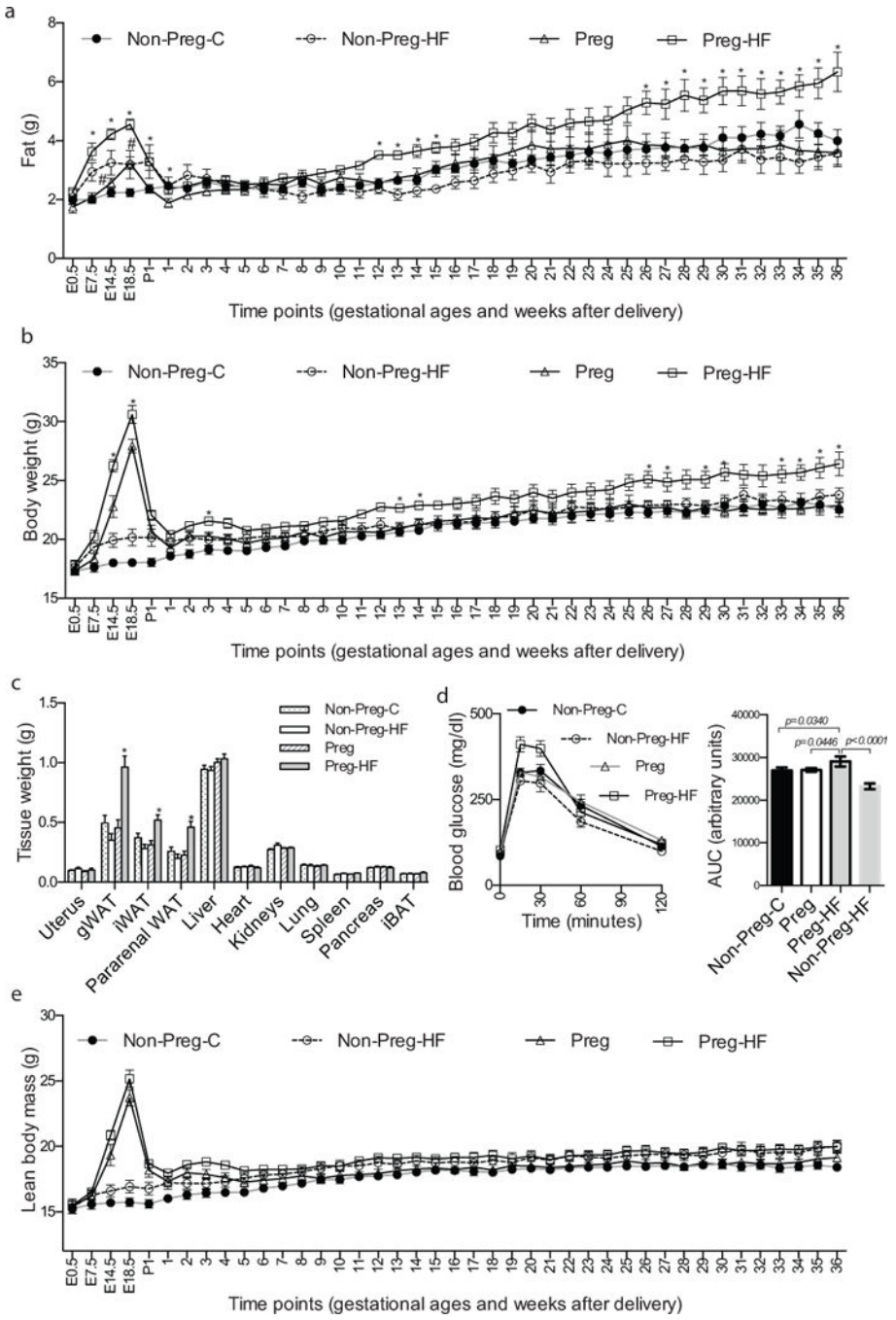

Figure 1. HFFDG induced EGWG and long-term postpartum obesity.

Nulliparous C57BL/6 female mice (10–12 weeks old) were mated with healthy or vasectomized males. Pregnancy was determined by the presence of a vaginal plug and assigned as embryonic day (E) 0.5. HF diet was provided to some pregnant mice (Preg-HF) or mice without pregnancy for 20 days (equal to mouse gestation, Non-Preg-HF). After delivery, regular chow was provided to all mice and lactation were stopped by removing pups. Body composition was monitored at indicate gestational ages and weekly after delivery using Echo-MRI (a,b&e). Tissues were weighed at the end of the study at fed state

(c). Glucose tolerance tested (GTT, i.p.) was performed 9-month after delivery with 6 hours fasting (d). Data are presented as mean ± SEM; n=16 for body composition study (a, b&e), n=8 for GTT (d); *p<0.05 vs. Preg at the same time point; # p<0.05 vs. the same group (Preg) at E0.5.

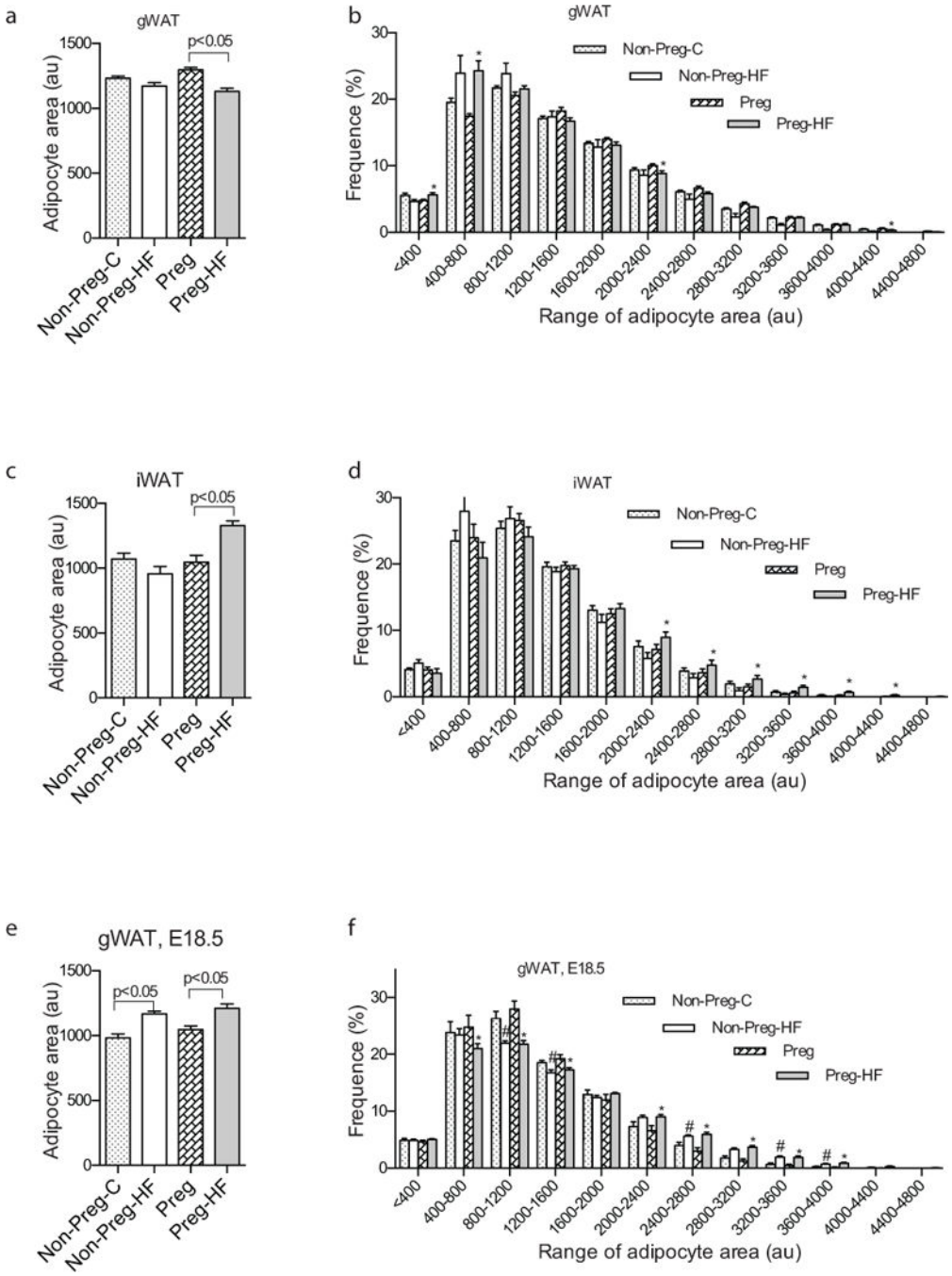

Figure 2. HFFDG programmed postpartum adiposity by inducing hyperplasia in visceral WAT and hypertrophy in subcutaneous WAT.

WATs were collected 9 months after delivery (a-d) or at E18.5 (e&f). Adipocyte area was measured by using ImageJ software with HE-stained tissue sections. Images (9 per section) were randomly selected. Data are presented as mean ± SEM; n=8; *$p<0.05$ *vs.* Preg, # $p<0.05$ *vs.* Non-Preg-C. au: arbitrary units.

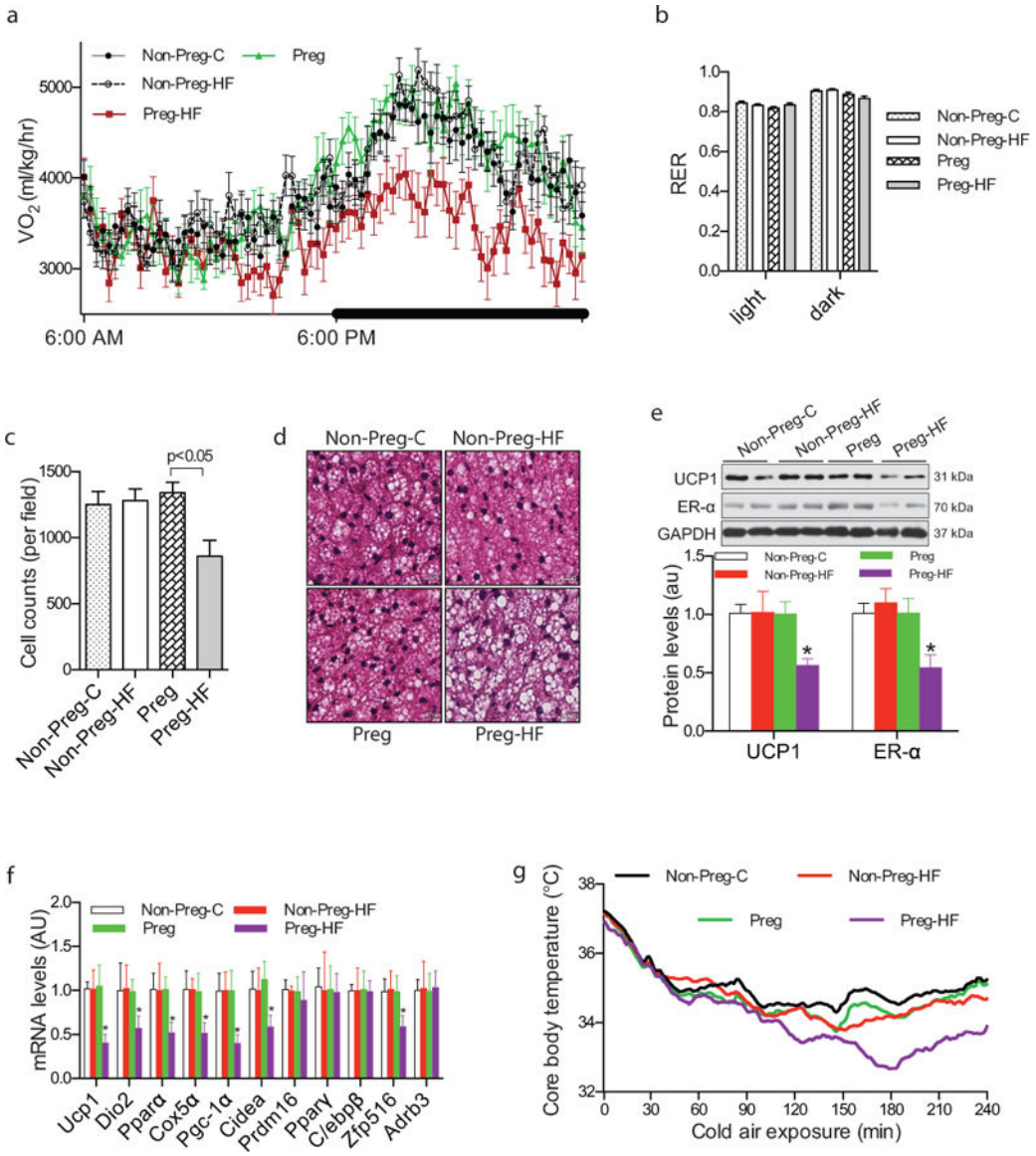

Figure 3. HFFDG reduced energy expenditure and BAT-mediated thermogenesis in late life of dams.

Eight months after delivery, energy expenditure rates (a, normalized by lean tissue mass) and RER (b) were studied by CLAMS. Adipocyte number (c) and tissue structure (d) of iBAT (9 months after delivery) were analyzed by counting nuclei with HE-stained section using ImageJ software. Expression levels of UCP1 and key players of brown adipogenesis were measured by Western blotting (e) and real-time PCR (f). Core body temperature (g) was monitored with i.p. implanted eMitter when mice were exposed to 4 °C air. Data are presented as mean ± SEM (a,b,c,e&f), except core body temperature (g, mean); n=8–10; *p<0.05 vs. Preg.

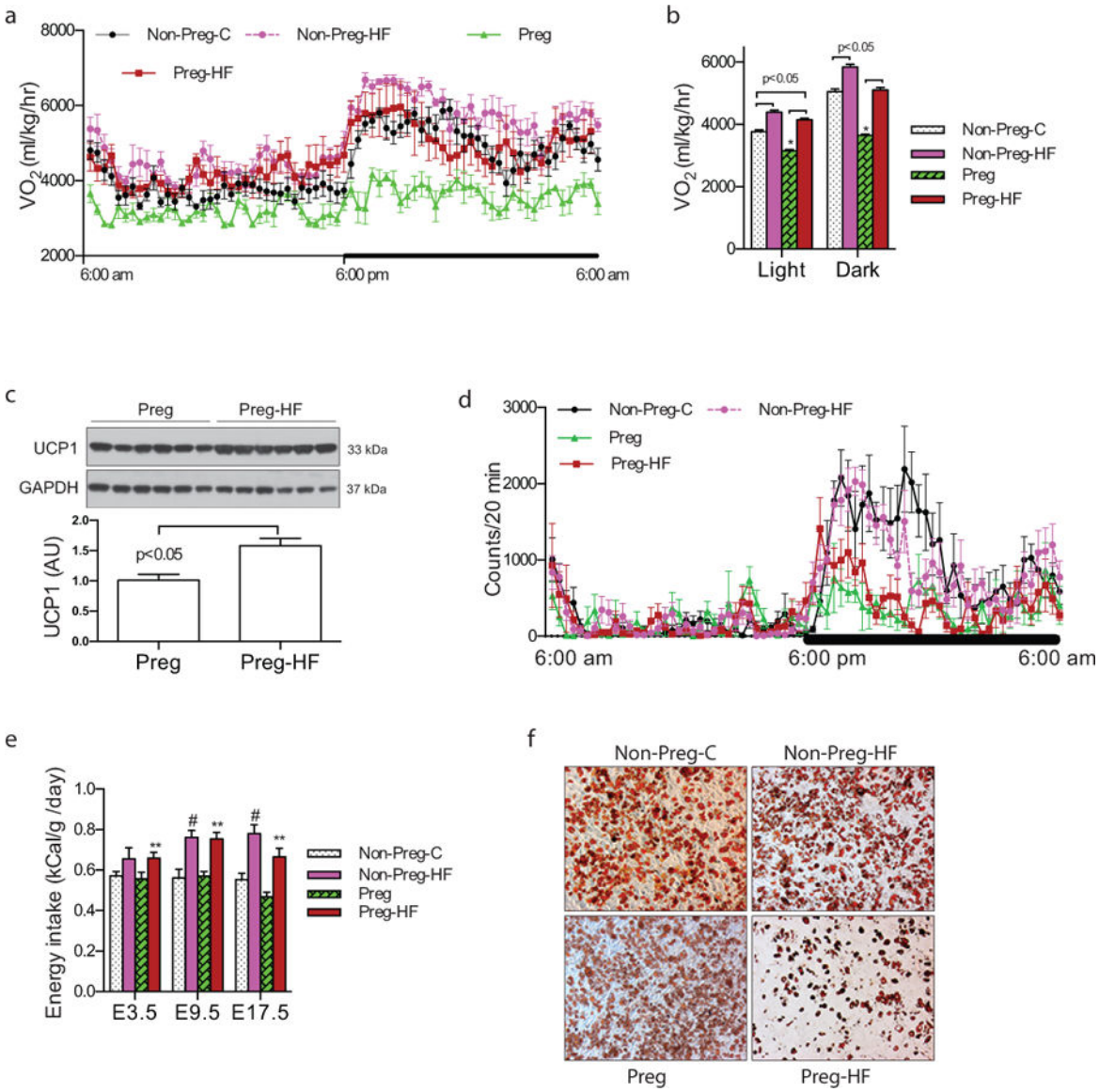

Figure 4. HF feeding reversed the inhibitory effect of gestation on energy expenditure and UCP1 expression in dam's iBAT.

C57BL/6 mice were fed with HF or regular chow during pregnancy or same duration without pregnancy. Energy expenditure rate (a&b) and locomotor activity (d) were measured using the CLAMS from E17.5 to E18.5. UCP1 protein levels of iBAT were compared between Preg and Preg-HF mice at E18.5 at room temperature (c). Food intakes were measured during CLAMS assay and normalized by energy density and body weight (e). The SVF from iBAT at E18.5 were differentiated into brown adipocytes and stained with oil red O (f). Data are presented as mean ± SEM; n=6–8, ** or #p<0.05 vs.* Non-Preg-C, ***p<0.01 vs. Preg.*

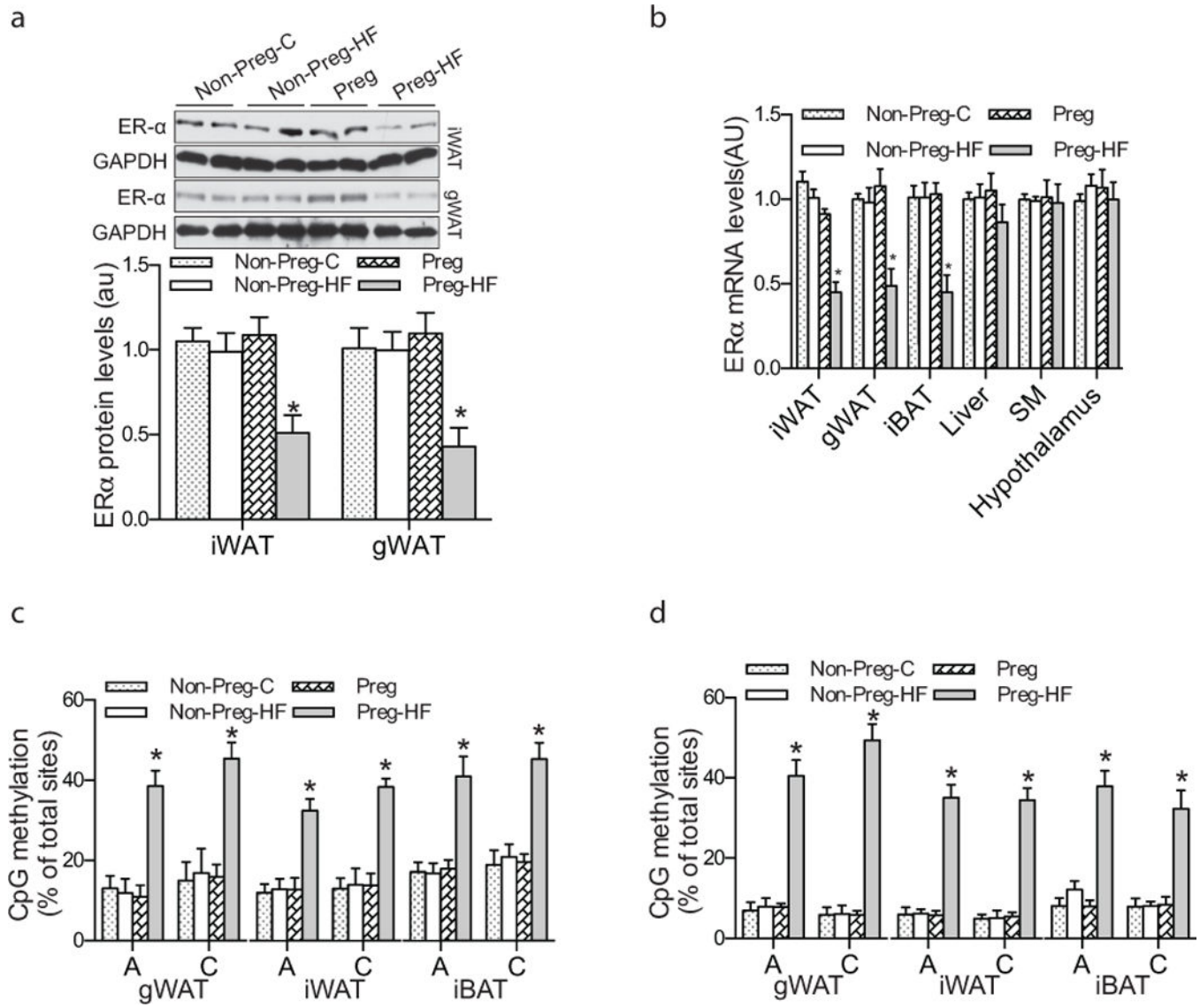

Figure 5. HFFDG increased ERα promotors methylation in adipocyte precursor cells and reduced ERα expression in WAT and iBAT in later life.

Expression of ERα at the protein (a) and mRNA (b) level was studied using tissue samples from mice 9 months after delivery. Methylation of CpG sites in the exon A and C of the ERα promoter was determined by using Pyrosequencing with tissue samples from mice 9 months after delivery (c) and adipocyte precursor cells from gWAT, iWAT and iBAT at E18.5 (d). N= 6, *$p<0.05$ *vs.* Preg. SM: skeletal muscle.

Table 1.

Sequences for real-time PCR primers

Gene	Forward (5’ to 3’)	Reverse (5’ to 3’)
18S rRNA	CGAAAGCATTTGCCAAGAAT	AGTCGGCATCGTTTATGGTC
Ucp1	TAAGCCGGCTGAGATCTTGT	GGCCTCTACGACTCAGTCCA
Dio2	GGAGCATCTTCACCCAGTTT	CTGCGCTGTGTCTGGAAC
Pparα	GGGCAGCTGACTGAGGAA	TCCGAGGGCTCTGTCATC
Cox5α	GTCCTTAGGAAGCCCATCG	TTAAATGAATTGGGAATCTCCAC
Pgc-1α	TGAAAGGGCCAAACAGAGAG	GTAAATCACACGGCGCTCTT
Cidea	AGGCCAGTTGTGATGACTAAGAC	AAACCATGACCGAAGTAGCC
Prdm16	CGTGGAGAGGAGTGTCTTCAG	ACAGGCAGGCTAAGAACCAG
Pparγ	CTGTGTCAACCATGGTAATTTCTT	TGCTGTTATGGGTGAAACTCTG
C/ebpβ	CACGTCTGTTGCGTCAGTC	TGATGCAATCCGGATCAA
Zfp516	TCTGCTTCACCTTCAGATGC	GTGTGGCAGATGCAGCAA
Adrb3	TCC CGA AGA AGG GAA CTG T	CCT TCC GTC GTC TTC TGT GT
Ebag9	GCAACAGTGTTCTCGTTCCTAA	GGGCAAAGTTATTTGATCTCCA