Sexually Dimorphic Role of G Protein-coupled Estrogen Receptor (GPER) in Modulating Energy Homeostasis

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ABSTRACT

The classical estrogen receptors, estrogen receptor-α and estrogen receptor-β, are well established in the regulation of body weight and energy homeostasis in both males and females, but the role for a G protein-coupled estrogen receptor 1 (GPER) as a modulator of energy homeostasis remains controversial. This study sought to determine whether gene deletion of GPER (GPER KO) alters body weight, body adiposity, food intake, and energy homeostasis in both male and female mice. Males and females exhibited different disruptions in energy homeostasis related to GPER function. These results provide new information elucidating a sexual dimorphism in GPER function in the development of postpubertal energy balance.

The main findings of this study are that there is a strong sexual dimorphism in the temporal onset of body weight gain in GPER KO mice. We also found that:

1) male GPER KO mice develop moderate obesity as they age and this is associated with reductions in energy expenditure, increased fat cell size, and increased lipid in brown adipose tissue;

2) female GPER KO do not differ with respect to adiposity when compared to WT mice initially, but over time as the mice age there is a divergence in body weight, with GPER KO females having increased body weight relative to WT females;

3) prior to the divergence of body weight, female GPER KO mice are less sensitive to modulators of food intake such as CCK and leptin;

4) ovariectomy induces weight gain in WT but not GPER KO mice and 17β-estradiol replacement was less affective in modulating body weight and glucose homeostasis in the GPER KO relative to WT mice;

5) central administration of 17β-estradiol to OVX WT mice actives pERK and did not do so in OVX GPER KO mice. These data indicate that females are less sensitive to the effects of estrogens to modulators of food intake and energy homeostasis.

RESULTS

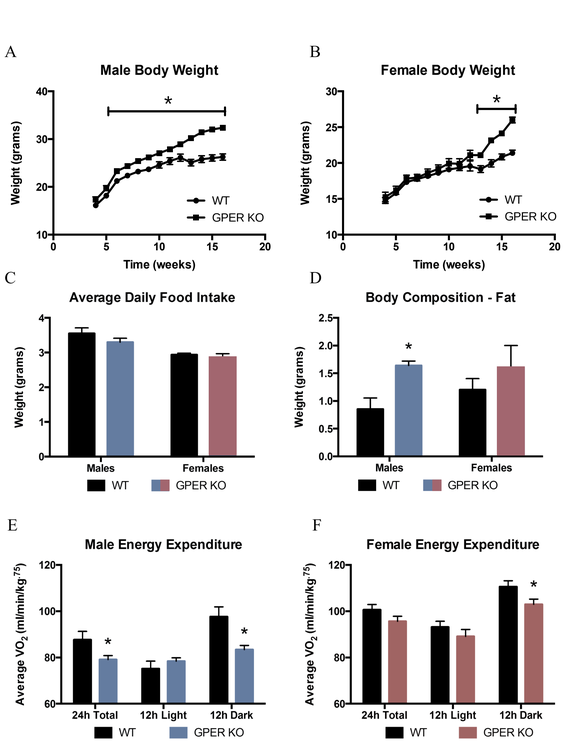

Figure 1. Body weight, food intake, and energy expenditure of GPER KO mice. **A.** Weekly body weights of male WT and GPER KO mice. **B.** Weekly body weights of female WT and GPER KO mice. **C**. Average daily food intake in male and female WT and GPER KO mice tracked at 13 weeks. **D.** Fat mass measured by NMR in 13 week old male and female WT and GPER KO mice. **E**. Energy expenditure was measured and represented by average VO2 for 10 week old male WT and GPER KO mice. F. Energy expenditure was measured and represented by average VO2 for 10 week old female WT and GPER KO mice. All data are expressed as mean ± SEM and n=12-19 per group. Values significantly different from sex- and age-matched WT controls are designated by asterisks above the column. * P<0.05.

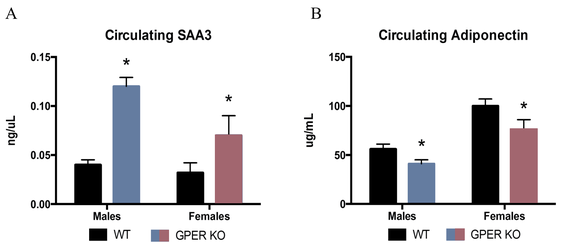

Figure 2. Circulating inflammatory factors and adipokines. **A**. Circulating SAA3 levels measured from serum in male and female 13 week old WT and GPER KO mice. **B.** Circulating adiponectin levels measured from serum in male and female 13 week old WT and GPER KO mice. All data are expressed as mean ± SEM and n=8-10 per group. Values significantly different from sex- and age-matched WT controls are designated by asterisks above the column. * P<0.05.

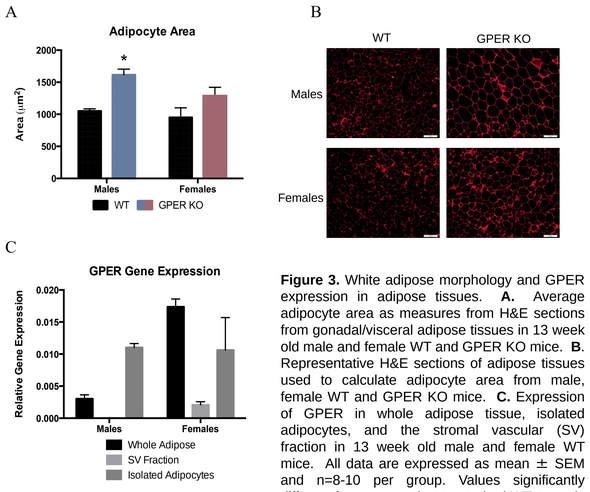

Figure 3. White adipose morphology and GPER expression in adipose tissues. **A.** Average adipocyte area as measures from H&E sections from gonadal/visceral adipose tissues in 13 week old male and female WT and GPER KO mice. **B**. Representative H&E sections of adipose tissues used to calculate adipocyte area from male, female WT and GPER KO mice. **C.** Expression of GPER in whole adipose tissue, isolated adipocytes, and the stromal vascular (SV) fraction in 13 week old male and female WT mice. All data are expressed as mean ± SEM and n=8-10 per group. Values significantly different from sex- and age-matched WT controls are designated by asterisks above the column. * P<0.05.

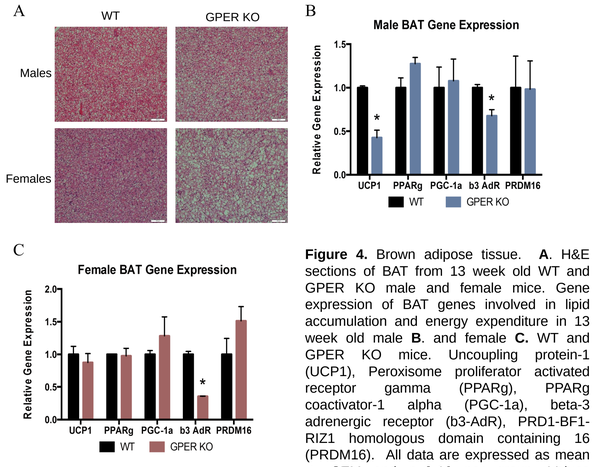

Figure 4. Brown adipose tissue. **A**. H&E sections of BAT from 13 week old WT and GPER KO male and female mice. Gene expression of BAT genes involved in lipid accumulation and energy expenditure in 13 week old male **B**. and female **C.** WT and GPER KO mice. Uncoupling protein-1 (UCP1), Peroxisome proliferator activated receptor gamma (PPARg), PPARg coactivator-1 alpha (PGC-1a), beta-3 adrenergic receptor (b3-AdR), PRD1-BF1-RIZ1 homologous domain containing 16 (PRDM16). All data are expressed as mean ± SEM and n=8-10 per group. Values significantly different from sex- and age-matched WT controls are designated by asterisks above the column. * P<0.05.

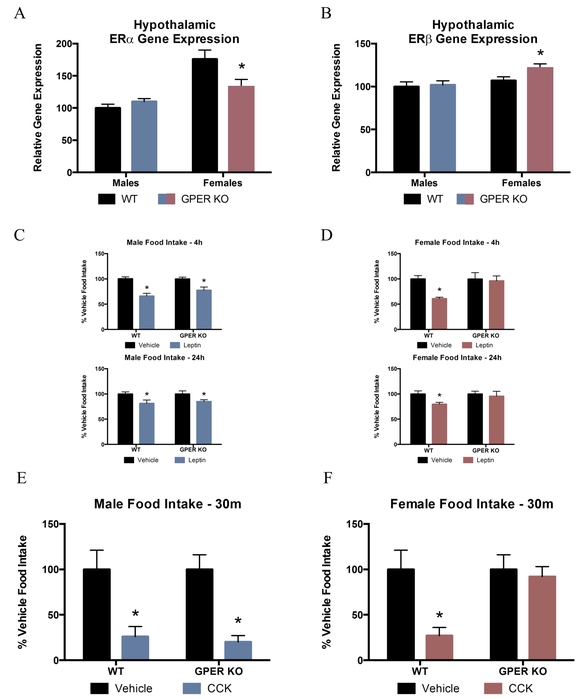

Figure 5. Expression of other ERs and effects of GPER on leptin and CCK sensitivity. **A.** Basal medial hypothalamic expression of ERα in 13 week old male and female WT and GPER KO mice. **B.** Basal medial hypothalamic expression of ERβ in 13 week old male and female WT and GPER KO mice. **C.** Average food intake of 13 week old male WT and GPER KO mice 4 and 24 hours after leptin administration. **D.** Average food intake of 13 week old female male WT and GPER KO mice 4 and 24 hours after leptin administration. **E**. Average food intake of 14 week old male WT and GPER KO mice 30 min after CCK administration. **F.** Average food intake of 14 week old female WT and GPER KO mice 30 min after CCK administration. All data are expressed as mean ± SEM and n=8-10 per group. Values significantly different from sex- and age-matched WT controls are designated by asterisks above the column. * P<0.05.

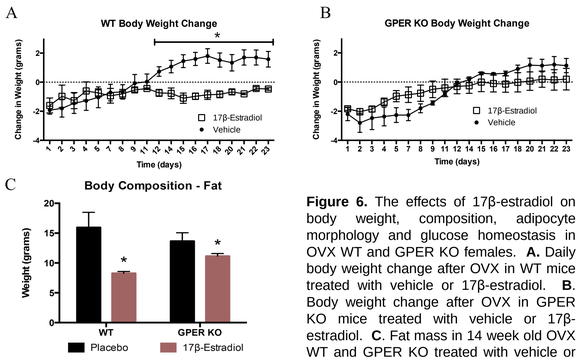

Figure 6. The effects of 17β-estradiol on body weight, composition, adipocyte morphology and glucose homeostasis in OVX WT and GPER KO females. **A.** Daily body weight change after OVX in WT mice treated with vehicle or 17β-estradiol. **B**. Body weight change after OVX in GPER KO mice treated with vehicle or 17β-estradiol. **C**. Fat mass in 14 week old OVX WT and GPER KO treated with vehicle or 17β-estradiol.

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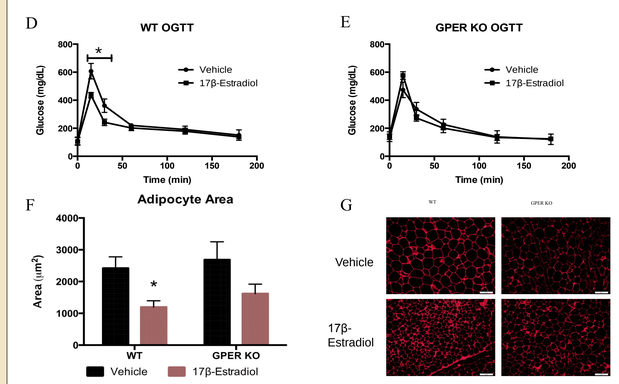

Figure 6 continued D. Oral glucose tolerance test in 13 week old OVX WT mice treated with vehicle or 17β-estradiol. **E**. Oral glucose tolerance test in 13 week old OVX GPER KO mice treated with vehicle or 17β-estradiol. **F**. Average adipocyte area as measures from H&E sections from visceral adipose tissues in 14 week old OVX WT and GPER KO mice treated with vehicle or 17β-estradiol. **G**. Representative H&E sections of adipose tissues measured in F. All data are expressed as mean ± SEM and n=8-12 per group. *P<0.05 compared with vehicle treatment.

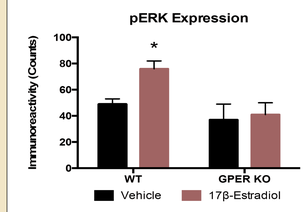

Figure 7. 17β-estradiol induced phosphorylation of ERK in the basal medial hypothalamus in WT and GPER KO OVX females. A single i3vt injection of 0.05 μg/μl 17β-estradiol increased pErk 1/2 in OVX WT but not OVX GPER KO females (Fig. 7). All data are expressed as mean ± SEM and n=5 per group. *P<0.05 compared with vehicle treatment.

CONCLUSION

In summary, we report a potential role for GPER in regulating body weight, body adiposity, and energy expenditure. The importance of estrogenic activity in regulating body weight in males has previously been demonstrated in the ERαKO mice [1], the aromatase knockout mice (ArKO) [2], as well as our recent findings with tissue specific knockdown of ERα [3,4]. Here, our data suggest strogenic activation of GPER in males and females may also be important for body weight regulation; however, the timing and tissues by which GPER is influencing has yet to be determined.

Therefore, we suggest a proposed model of how GPER affects energy balance.

In males: ERα and ERβ appear to be important in modulating energy homeostasis and our data suggest GPER is another critical player. It is possible that as males have lower circulating levels of estrogens and lower levels of estrogen receptors, perturbations in one or more ERs critically impacts energy expenditure and adipose tissue function and morphology. These findings would be consistent with a recent publication by Finkelstein et al.[5], where they demonstrate an important role for estrogens in adipose tissue of men.

In females: It is possible deletion of GPER has less of an impact due to compensatory responses from the other estrogen receptors. Importantly, female GPER KO mice do not differ in body weight or adiposity when compared to WT mice, but over time there is a divergence in body weight with GPER KO females having increased body weight relative to WT females. Prior to the divergence in body weight, female GPER KO mice are less sensitive to modulators of food intake: cholecystokinin (CCK) and leptin. Additionally, ovariectomy induces weight gain in WT and not GPER KO mice, and 17β-estradiol replacement was less affective in modulating body weight and glucose homeostasis in the GPER KO relative to WT mice. Central administration of 17β-estradiol to OVX WT activated pERK but failed to do so in OVX GPER KO mice suggesting, reduced 17β-estradiol sensitivity in the female GPER KO.