Beta-3 adrenergic receptor activation induces white adipose tissue browning in a *sex* and *depot*-dependent manner.

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Abstract

Female cardiometabolic health (e.g., glucose tolerance, body composition) is protected compared to males when fed high-fat-diet (HFD). Activation of the beta-3 adrenergic receptor (β3AR) via the chemical ligand CL316,243 (CL) induces browning in white adipose tissue (WAT), a process sufficient to improve glucose tolerance and reduce visceral adiposity. ***Our aim was to determine (a) if sex differences exist in CL-induced WAT browning; and (b) if two WAT depots, perigonadal (PGAT) and subcutaneous (SQAT), differ in CL responsiveness, in a sex-specific manner.*** To this end, 8-week-old male and female mice, bred on a C57BL/6J background were fed HFD for a total of 16 weeks, and given daily CL injections (1ug/g body weight) for the final 2 weeks. We compared those groups using 2x2 ANOVA to determine main and interaction effects (S=sex; T=treatment; SxT=interaction) for browning and adipocyte health markers in PGAT and SQAT (gene expression via qrtPCR; protein expression via Western blot). We report here that the effects of CL are both depot-dependent and sex-specific. As expected, females had greater uncoupling protein 1 (UCP1); however, this was only observed in PGAT (S, p<0.001), not SQAT. UCP1 was more responsive in female PGAT (SxT, p=0.011); however, sexes were equally responsive in SQAT. Interestingly, in male SQAT, PGC1a (i.e., mitochondrial biogenesis marker) responded significantly better to CL (SxT, p=0.046); whereas in PGAT, females were more responsive (SxT, p=0.026). We also show for the first time that CL increases glucose-related protein 75 (GRP75), a known mitochondrial translocator protein, in both depots, in both sexes (T, p<0.05, both). Lastly, while female PGAT had greater adiponectin, males had higher adiponectin in SQAT (S, p<0.05, both). In conclusion, it appears SQAT is more responsive to CL in males, whereas PGAT is more responsive in females, which we hypothesize is partially explained by differences in local estrogen exposure.

Methods

Statistical Analysis:

For the 4 groups analyzed a **2x2 ANOVA** was performed using SPSS 27.0.1 to determine the following:

- Main Effects of **Sex (S)** or **Treatment (T)**
- Interaction of **Sex and Treatment (SxT)**

For all statistical tests, significance was accepted as p<0.05.

Background & Significance

- Female mice are protected against high-fat-diet (HFD) induced metabolic dysfunction. We confirmed this by showing that, compared to males, females have lower:
  - ✓ Body weight (S, p<0.001) **(Fig. 1a and 1b),**
  - ✓ WAT pad weight (S, p=0.000) **(Fig. 1c and 1d),**
  - ✓ % fat mass (S, p=0.018) **(Fig. 1e),**
  - ✓ GTT area-under-curve (AUC) (S, p=0.000) **(Fig. 1f and 1g),** and
  - ✓ Fasting glucose **(Fig. 1h).**
- We confirmed that activation of the sympathetic nervous system specifically in adipocytes through the beta-3 adrenergic receptor (β3AR) via the chemical ligand *CL316,243 (CL)* is sufficient to:
  - ✓ Decrease body weight (T, p=0.037) **(Fig. 1b),**
  - ✓ Reduce visceral adiposity (T, p=0.001) **(Fig. 1c),** and,
  - ✓ Improve glucose tolerance (T, p=0.000; T=0.007) **(Fig. 1g and 1h).**

Hypotheses

***(a)* Female WAT (PGAT or SQAT) will be more sensitive to CL-induced browning.**

***(b)* The PGAT depot will be more responsive to CL in females, compared to SQAT, but there will be no differences in response to CL in male WAT depots (PGAT and SQAT).**

Results

- The beneficial effects of CL on *body composition* and *glucose homeostasis* are *non-sex-specific* (i.e., females and males respond equally to CL) (SxT, p>0.05) **(Fig. 1)**.
- Our data indicate that PGAT pad weight (T, p=0.001) **(Fig. 1c)** is more sensitive to the effects of CL compared to SQAT (T, p=0.153) **(Fig. 1d).**
- We show that the effects of CL on mitochondrial protein expression are *depot-dependent* and *sex-specific*, such that:
  - ❑ Females had greater uncoupling protein 1 (UCP1); however, this was only observed in PGAT (S, p<0.001), not SQAT (S, p>0.05) (**Fig. 2a).**
  - ❑ Female PGAT was more responsive to CL-induced UCP1 expression (SxT, p=0.011); however, sexes were equally responsive in SQAT (SxT, p=0.444) **(Fig. 2a)**.
  - ❑ PGC1a protein expression responded significantly better to CL in male SQAT (SxT, p=0.046); whereas in PGAT, females were more responsive (SxT, p=0.026) **(Fig. 2b)**.
  - ❑ Male PGAT had lower OXPHOS protein compared to female PGAT (S, p=0.010), whereas there was no sex effect in SQAT; furthermore, CL increased total OXPHOS protein expression in both PGAT and SQAT (T, p>0.05, both).
- We show for the first time that CL increases glucose-related protein 75 (GRP75), in both depots, in both sexes (T, p<0.05, both) **(Fig. 2d)**.
- Lastly, while female PGAT had greater adiponectin, males had higher adiponectin in SQAT (S, p<0.05, both) **(Fig. 2e),** and CL increased leptin expression only in PGAT (T, p=0.016) **(Fig. 2f).**

Conclusions

❖ CL improves HFD-induced glucose intolerance and body composition in both sexes!

❖ WAT responds to CL differently in males and females, however, this is *depot-dependent.*

❖ PGAT appears to be the major site of browning in females, however, SQAT is the major site of browning in males.

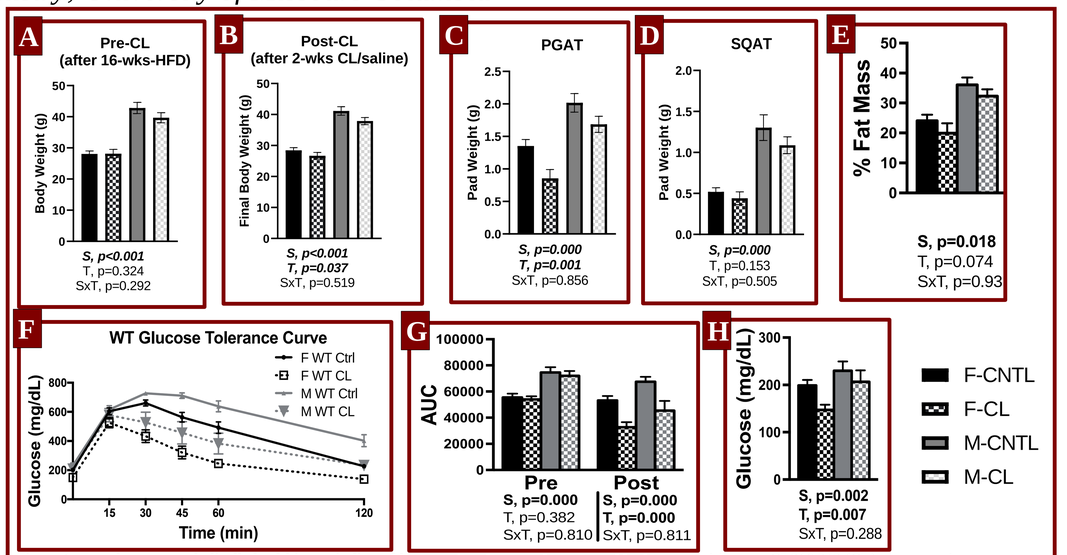

Figure 1: Females are metabolically healthier, as indicated by superior glucose tolerance and body composition, yet CL316,243 (CL) treatment improves high-fat-diet (HFD)-induced changes in body composition and glucose intolerance equally in both sexes. 22-week-old male and female mice were given daily injections of CL *(1ug/g body weight)* for two weeks, after being fed a HFD for 16 weeks, and compared to control *(saline)*. The effect of sex and CL on body composition and glucose tolerance was evaluated and presented here. (a) body weight after 16 weeks of HFD, pre-CL treatment; (b) body weight 2 weeks post-CL treatment; (c) perigonadal adipose tissue (PGAT) depot weight; (d) subcutaneous adipose tissue (SQAT) depot weight; (e) percent fat mass; (f) glucose tolerance test (GTT) curve post-CL treatment; (g) area-under-curve (AUC) for GTT for both pre- and post- CL treatment; (h) fasting blood glucose post CL-treatment. All data are presented as mean ± SEM; n = 3-15/group. Statistical differences were determined using a 2x2 ANOVA; main effects of sex (S) and treatment (T), as well as interactions between sex and treatment (SxT) are given below each graph; significance was accepted as p<0.05.

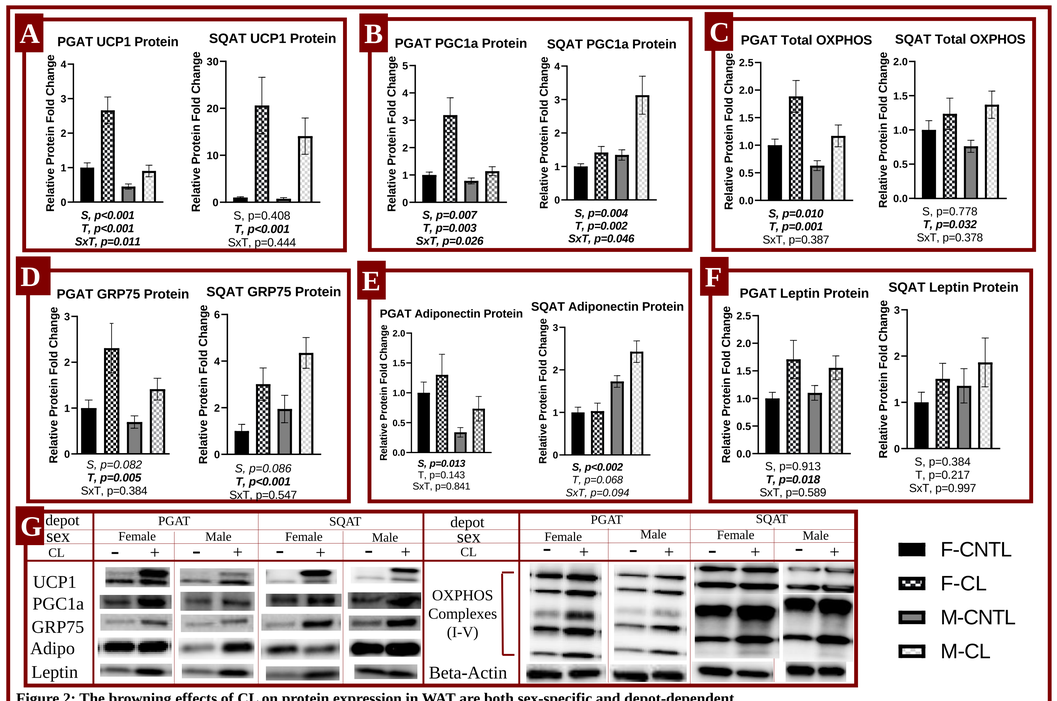

Figure 2: The browning effects of CL on protein expression in WAT are both sex-specific and depot-dependent.
22-week-old male and female mice were given daily injections of CL for two weeks, after being fed a HFD for 16 weeks. Perigonadal adipose tissue (PGAT) and subcutaneous adipose tissue (SQAT) depots were collected from mice at sacrifice, and protein expression was measured via western blotting. The effect of sex and CL on classical (e.g. UCP1, PGC1a, OXPHOS) and modern (e.g. GRP75) markers of WAT browning were assessed in both PGAT and SQAT. (a) Uncoupling Protein 1 (UCP1); (b) PPAR Gamma Coactivator 1 alpha (PGC1a); (c) total oxidative phosphorylation complexes (OXPHOS); (d) Glucose Related Protein 75 (GRP75); (e) Adiponectin; (f) Leptin; (g) representative images for each western blot. All proteins of interest were normalized to beta-actin and all data are presented as mean ± SEM; n=7-9/group. Statistical differences were determined using a 2x2 ANOVA; main effects of sex (S) and treatment (T), as well as interactions between sex and treatment (SxT) are given below each graph; significance was accepted as p<0.05.