Beta3-Adrenergic Receptor (B3AR) Activation Induces Estrogen Receptor Beta (ERB) and Improves Mitochondrial Metabolism in Adipocytes

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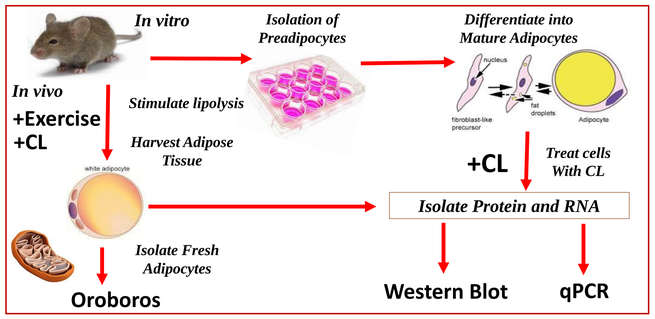
Abstract

In vivo, estrogen receptor beta (ERβ) activation protects against obesity and white adipose tissue (WAT) dysfunction. Mechanisms are unknown, but we hypothesize that it involves adipocyte-specific mitochondrial activity via induction of OXPHOS and UCP1– proteins that characterize WAT "browning," a process *sufficient* to rescue metabolic dysfunction. In cancer cells, increasing mitochondrial ERβ increases OXPHOS transcription and mitochondrial activity via a mechanism requiring mitochondrial transporter, GRP75. We sought to determine whether this mechanism applies to adipocytes *in vitro*, and the potential *in vivo* implications. In wild type (WT) mice, the adipocyte-specific B_3AR ligand, CL316,243 (CL) induced WAT UCP1 and ERβ protein (*Western blot*), and GRP75 mRNA (*qPCR*) (all n=10/grp, P<0.05). In primary adipocytes from WT mice (n=3/treatment), CL increased UCP1 (~2-fold; P<0.05) and ERβ protein (~4-fold; *trend*, P=0.097). *In vivo*, we show that adipocytes harvested from mutant mice missing ERb DNA binding domain ($ERβ_{DBD}KO$) display suppressed mitochondrial O_2 consumption (*via Oroboros*; n=6-8/grp, P<0.05). We next tested whether $ERβ_{DBD}$KO mice would have impaired exercise-induced WAT browning. Remarkably, unlike WT (~3-fold UCP1 increase; P<0.05), $ERβ_{DBD}$KO mice were nonresponsive to exercise induction of UCP1 protein in WAT. Taken together, our data support a critical role for adipocyte ERβ in mediating lipolysis driven mitochondrial responses. We hypothesize that the mechanism involves GRP75. Identifying the mechanism by which ERβ enhances adipocyte mitochondrial activity has enormous potential to treat a spectrum of metabolic diseases.

Methods



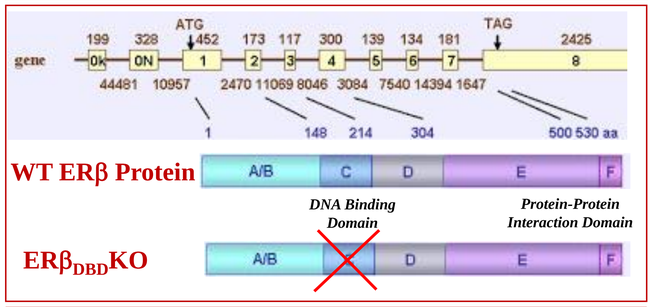
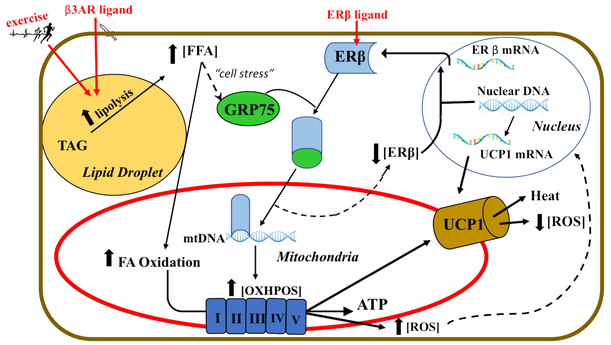


Figure 1. Hypothetical Mechanism Exercise or CL-induced activation of the β3 adrenergic receptor →increased [FFA] →induction of GRP75, a known heat shock and mitochondrial transporter protein → recruitment of ERβ to the mitochondria. It is this mitochondrial sequestration of ERβ that drives the increase in total ERβ. In the mitochondria, ERβ increases the transcription of the mitochondrial encoded OXPHOS subunits, facilitating the increased demand for FA oxidation. The increase in aerobic metabolism leads to production of reactive oxygen species (ROS), which triggers the nuclear transcription and mitochondrial localization of UCP1, known to buffer ROS production.



Background

- Adipocyte metabolic dysfunction has been implicated in several diseases.
- Estrogen receptor alpha (ERα) is known to protect adipose tissue (AT) from insulin resistance **(Figure A)** but the role of ERβ within AT is not well understood.
- The B3-adrenergic receptor agonist, CL314,243 (CL) leads to induction of UCP1 **(Figure B)**, a process sufficient to rescue AT insulin resistance **(Figure C).**
- The ERα knock-out (ERαKO) model is more susceptible to CL-mediated UCP1 induction; the mechanism is unknown but may involve ERβ.
- In the ERαKO, ERβ may protect against OVX-induced obesity and adipocyte dysfunction **(Figure D)**
- *In vivo*, CL increases the expression of ERβ **(Figure E)**, implicating ERβ as a potential mechanism by which CL induces UCP1 and rescues metabolic dysfunction.

Study Aim: Determine the role played by ERβ in lipolysis-mediated induction of UCP1 and subsequent improvements in adipocyte mitochondrial function. The *hypothesis* is that stimulation of lipolysis by both exercise and CL will lead to an increase in UCP1, ERβ and the the mitochondrial transporter protein, GRP75 (*see Fig. 1*).

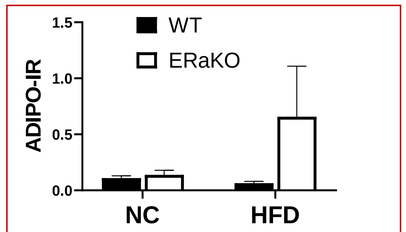


Figure A: ERa Ablation Exacerbates HFD Induced Adipocyte Dysfunction
WT and ERaKO mice were feed Normal Chow (NC) or High Fat Diet (HFD) and adipocyte insulin resistance (ADIPO-IR) was measured. Data expressed as mean ± standard error of the mean (SEM); n = 4-10/group.

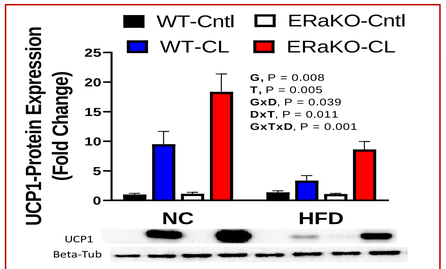


Figure B: CL Induces UCP1 in SQAT in ERaKO and WT mice
HFD and NC feed mice were treated with CL for 2 weeks. UCP1 protein expression was measured using Western Blot analysis and normalized to beta-tubulin. Data are expressed as mean ± standard error of mean (SEM); n = 4-10/group; Significance for main effect or interaction was accepted as P < 0.05. G=main effect of genotype; T=main effect of treatment; GxD=interaction between genotype and diet; DxT=interaction between diet and treatment; GxTxD=interaction between genotype, treatment and diet.

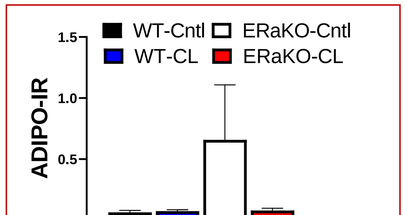


Figure C: CL Rescues HFD induced Dysfunction
WT and ERaKO mice were fed HFD for 10 weeks followed by CL treatment for 2 weeks. Adipocyte insulin resistance (ADIPO-IR) was assayed as a measure for adipocyte metabolic dysfunction. Data are expressed as mean ± standard error of mean (SEM); n = 10 group.

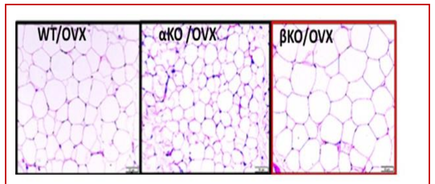


Figure D: ERb is Protective against OVX-induced Adiposity
WT, ERaKO and ERbKO mice were ovariectomized and their WAT was examined by staining. While knocking out ERb exacerbates OVX induced adiposity, it appears that in the ERaKO, were there is enhanced ERb signaling-ERb is protective.

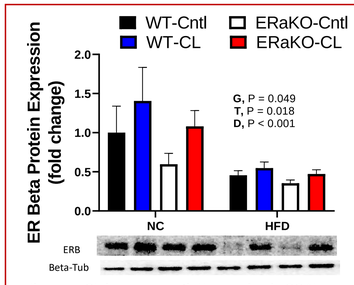


Figure E: CL increases ERb Expression in SQAT
WT and ERaKO mice were feed Normal Chow (NC) or High Fat Diet (HFD) for 10 weeks then treated with CL for 2 weeks. ERb expression in the SQAT depots was measured by western blot analysis. Data are expressed as mean ± standard error of mean (SEM); n = 4-10/group; statistical significance was accepted as P < 0.05. G=main effect of genotype; T=main effect of treatment; D=main effect of diet.

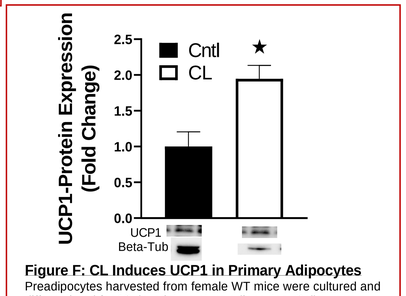


Figure F: CL Induces UCP1 in Primary Adipocytes
Preadipocytes harvested from female WT mice were cultured and differentiated for 10 days into mature adipocytes. Cells were treated with 1uM CL for 24 hours followed by western blot analysis; blots were normalized to beta-tubulin. Data are expressed as mean ± standard error of mean (SEM); n = 3/group. Stastical significance was accepted at P < 0.05 by t-test and indicated by ★ above the bar.

Results

- We demonstrate for the first time, ***in vitro* that CL increases UCP1 and ERβ protein expression (Figure F and G).**
- *In vivo*, like CL (i.e., chemical induction of lipolysis), exercise (EX) induces AT UCP1 **(Figure H)**, but not in the $ERβ_{DBD}$KO, demonstrating that **ERβ is required for EX-induced UCP1**, and thus may be critical for lipolysis-mediated adaptations in adipocyte metabolism.
- $ERβ_{DBD}$KO mice have suppressed mitochondrial O_2 consumption compared to WT, whereas CL increases mitochondrial respiration in both genotypes **(Figure I).**
- *In vivo*, in addition to increasing mitochondrial activity and UCP1, CL increases gene **(Figure J)** and protein **(Figure K)** expression of WAT GRP75.

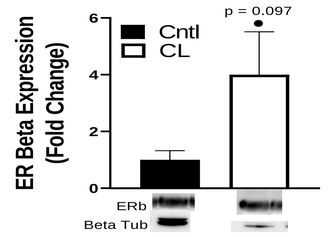


Figure G: CL Increases ERb in Primary Adipocytes
Preadipocytes harvested from female WT mice were differentiated for 10 days into mature adipocytes and treated with 1uM CL for 24 hours followed by western blot analysis, bands were normalized to beta tubulin. Data are expressed as mean ± standard error of mean (SEM); n = 3/group. Stastical significance was accepted as P < 0.05 by t-test. ● indicates a stastical trend.

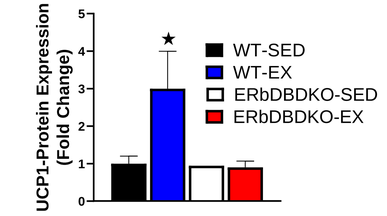


Figure H: ERb is Required for EX-Mediated Browning
Female WT and ERbDBDKO mice were fed a HFD and OXV at 14-15 weeks of age. Upon recovery they were split into two groups: sedementary (SED) or Exercise (EX). SED mice had a locked wheel, whereas EX mice had voluntary wheel running. Treatment lasted for 8 weeks. UCP1 protein expression within WAT was measured by western blot analysis, bands were normalized to beta tubulin. Data expressed at mean ± standard error of mean (SEM); n=10/group. Stastistical significance was accepted as P < 0.05 by 2-way ANOVA and indicated by ★.

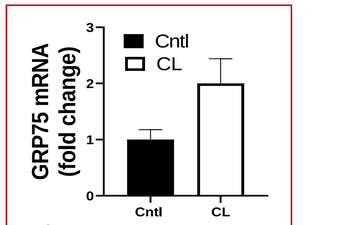


Figure J: CL Increases GRP75 mRNA in vivo
Male and Female WT mice were feed a HFD for 10 weeks followed by CL treatment for 2 weeks. GRP75 mRNA expression was measured in WAT using RT-PCR. Data are expressed as mean ± standard error of mean; n = 9-10/group.

Conclusions

- CL significantly upregulates UCP1, mitochondrial O_2 consumption, and protein levels of ERβ and GRP75.
- $ERβ_{DBD}$KO mice have suppressed basal mitochondrial O_2 consumption, yet are *more susceptible* to CL-mediated increases in O_2 consumption.
- **Future studies will:**
  - Determine if this is due to an increase in an alternative ERβ sliceform with a more pronounced C-terminal protein interaction domain;
  - Determine the subcellular (i.e., mitochondrial vs. cytosolic) localization of ERβ in response to CL;
  - Further interrogate the molecular mechanism by studying the effects of CL in the absence of ERβ and/or GRP75 using molecular techniques to knockdown respective genes.

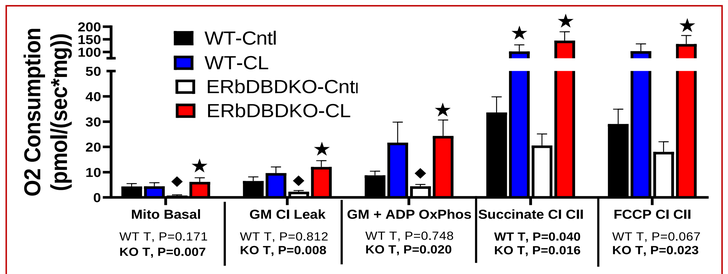


Figure I: ERbDBDKO Mice have Impaired Mitchondrial O2 Consumption
Freshly isolated mitochondia from male and female WT and ERbDBDKO mice were analyzed via Oroboros to measure O2 consumption. Data are expressed as mean ± standard error of mean (SEM); n = 6-8/group. Stastical significance was accepted as P < 0.05 by t-test. ◆ indicates main effect of genotype. . The succinate group and FCCP group were trending toward significant genotype effects, with P = 0.1 and P = 0.15, respectively. Significant main effects of treatment are indicated by ★ and corresponding P values displayed below each group.

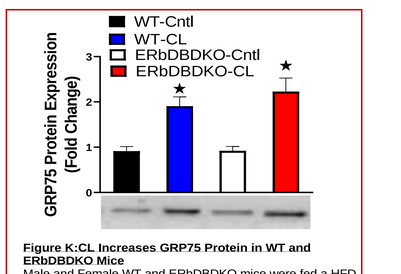


Figure K:CL Increases GRP75 Protein in WT and ERbDBDKO Mice
Male and Female WT and ERbDBDKO mice were fed a HFD for 10 weeks followed by CL treatment for 2 weeks. GRP75 protein expression within WAT was measured using western blot analysis. Data are expressed as mean ± standard error of mean. n = 16-17/group. Statistical significance was accepted as P < 0.05 and indicated by ★.

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