(PL-02) - A Significant Role of Estrogenic Receptor-α in the Cardiomyocyte Mitochondrial Regulation in Ovariectomized Rats

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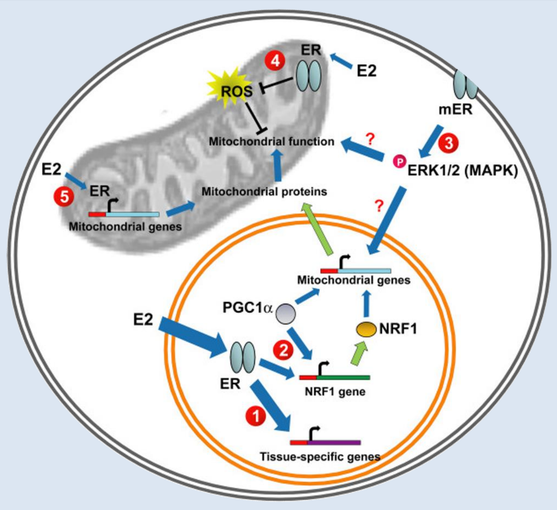
Introduction

The mitochondria regulate the estrogenic biosynthesis, and the estrogen (E2) hormone is implicated in controlling the mitochondrial function and homeostasis through its estrogenic receptors (ERs) in most cell types.

However, its direct mechanisms and targets still need further elucidation. The ERβ is currently believed to be responsible for most estrogen's mitochondrial function.



Molecular targets of estrogen in regulating mitochondrial function.

Aim of Work

This study aimed at investigating the molecular relationship between the mitochondria, E2, and its receptors in cardioprotection after menopause using the selective estrogenic receptor downregulator, fulvestrant (F).

Materials and Methods

Sixty-four female Wistar rats (200-220 g) were randomly divided into **two groups**. The first group (n=32) was **sham-operated** and allowed to recover for 4 weeks and then subdivided into 4 subgroups (n=8), while the second group (n=32) underwent a **bilateral ovariectomy**, to disturb the female sex hormones signaling axis, and after 4 weeks of recovery, ovariectomized rats were subdivided into 4 subgroups (n=8).

Experimental Groups

- **Sham + Vehicle**: • An oral daily dose of the ***vehicle, corn oil, i.p., for 28 days***.
- **Sham + E**: • Rats received estradiol (E) at a dose of ***(30 µg/kg, i.p.) for 28 days***
- **Sham + F**: • Rats received ***fulvestrant (F)*** at a dose of , ***(5 mg/kg, i.p.), for 28 days***
- **Sham + E + F**: • Rats received a ***combination*** of both drugs ***(E2+F)*** at the former doses ***for 28 days***.
- **OVX + Vehicle**: • An oral daily dose of the ***vehicle, corn oil, i.p., for 28 days***.
- **OVX + E**: • Rats received estradiol (E) at a dose of ***(30 µg/kg, i.p.) for 28 days***
- **OVX + F**: • Rats received ***fulvestrant (F)*** at a dose of , ***(5 mg/kg, i.p.), for 28 days***
- **OVX + E + F**: • Rats received a ***combination*** of both drugs ***(E2+F)*** at the former doses ***for 28 days***.

Blood sampling & Sacrifice → **Serum Samples**, **Heart**

	Parameters
Cardiac Mitochondrial Mitophagy	Beclin-1, LC3B, DRP1, PINK1, MNF2
Cardiac Mitochondrial Biogenesis	AMPK, PGC1-α, PGC1-β, NRF1, TFAM, mtDNA
Serum Cardiac Profile	ANP, Troponin1, CK-MB, NOx
Serum Lipid Profile	TG, LDL-c, HDL-c, Cholesterol, TG

Results

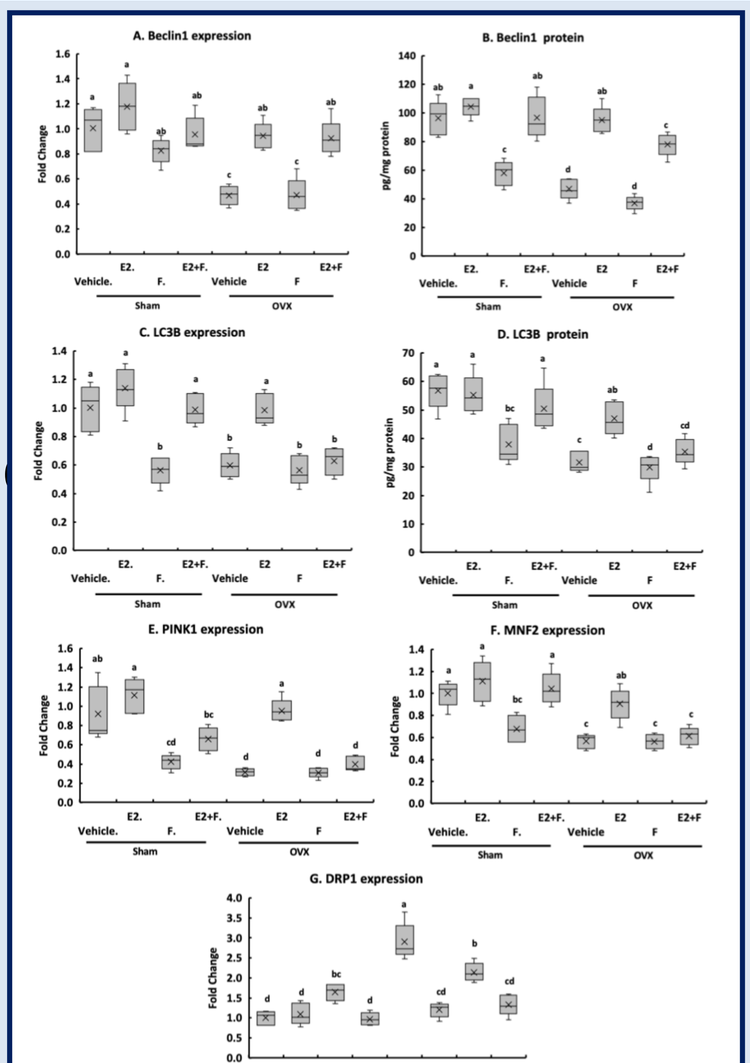


Figure 1: Effect on Cardiac Mitophagy

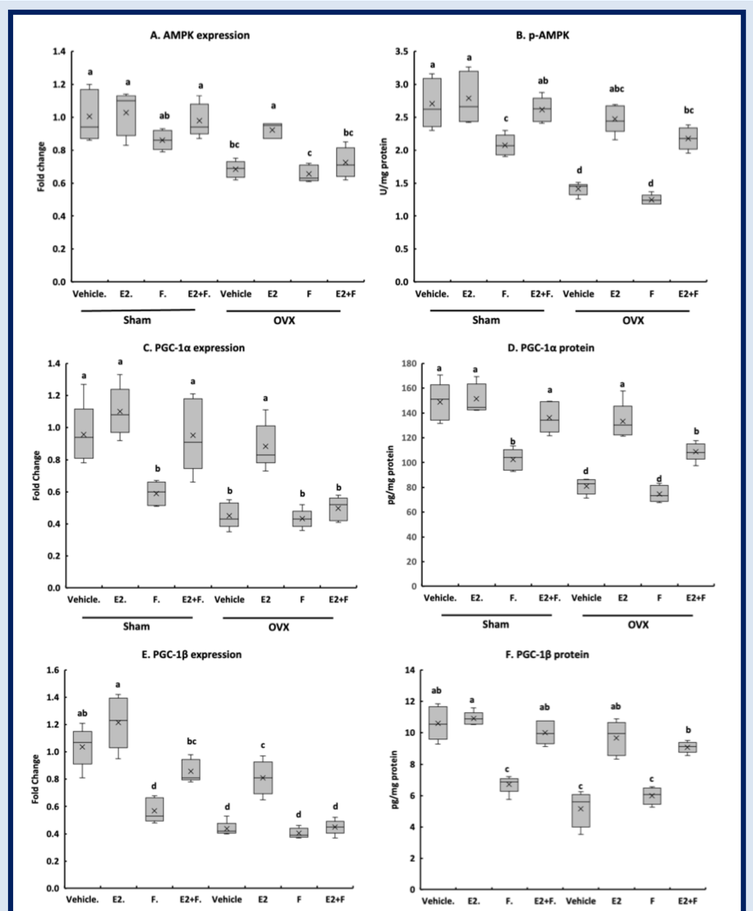


Figure 2: Effect on Cardiac AMPK-related Mitochondrial Biogenesis

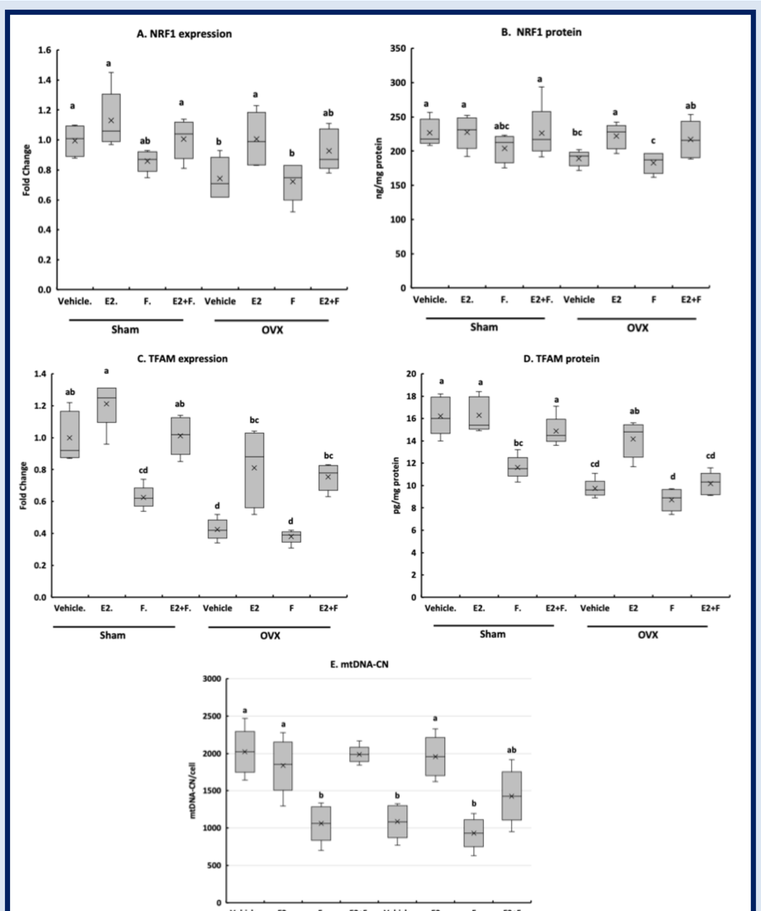


Figure 3: Effect on Cardiac Mitochondrial Biogenesis.

Comparisons among groups were analyzed using the one-way ANOVA test followed by the Tukey post hoc test. Data are compared at $p< 0.05$ with the vehicle, E2; estradiol, F; fulvestrant, and E2+F. Values are presented as means ± SD (n=8). OVX; ovariectomized rats. Means with common letters are not significant (i.e. means with different letters are significant)

		TG (mg/dl)	Cholesterol (mg/dl)	c –HDL (mg/dl)	c –LDL (mg/dl)	TG- Myocytes (mg/g tissue)
Sham	Vehicle	$49.4^{c} \pm 6.7$	$116^{c} \pm 8.73$	$45.6^{ab} \pm 1.14$	$60.3^{bc} \pm 9.06$	$28.4^{d} \pm 3.56$
	E2	$48.4^{c} \pm 9.2$	$109^{d} \pm 11.4$	$47.8^{a} \pm 2.59$	$51.3^{c} \pm 8.1$	$31.6^{d} \pm 2.30$
	F	$125^{a} \pm 9.2$	$157^{ab} \pm 19.2$	$39.2^{bc} \pm 3.8$	$93.2^{ab} \pm 18.4$	$44.6^{b} \pm 2.88$
	E2+ F	$61.4^{c} \pm 10.4$	$137^{abc} \pm 12.5$	$45.8^{ab} \pm 2.05$	$78.7^{ab} \pm 12.6$	$34.8^{cd} \pm 5.02$
OVX	Vehicle	$138^{a} \pm 7.4$	$158^{ab} \pm 10.6$	$38.5^{bc} \pm 5.09$	$91.6^{ab} \pm 15.1$	$50.4^{ab} \pm 4.39$
	E2	$85.1^{b} \pm 6.4$	$127^{bc} \pm 15.8$	$46.4^{a} \pm 3.4$	$65.6^{bc} \pm 15.3$	$36.8^{cd} \pm 4.63$
	F	$145^{a} \pm 12.5$	$166^{a} \pm 12.6$	$36.4^{c} \pm 3.68$	$100^{a} \pm 13.2$	$55.8^{a} \pm 7.85$
	E2+ F	$124^{a} \pm 19.7$	$162^{a} \pm 16.7$	$38.8^{bc} \pm 3.35$	$98.7^{a} \pm 21.3$	$46.8^{ab} \pm 2.77$

Table 1: Serum lipid profile and TG-Myocytes

		ANP (ng/ml)	CK-MB (U/l)	Troponin-I (ng/ml)	NOx (µmol/mg)
Sham	Vehicle	$0.330^{ab} \pm 0.051$	$12.4^{f} \pm 1.10$	$0.170^{c} \pm 0.015$	$45.5^{a} \pm 3.56$
	E2	$0.344^{a} \pm 0.029$	$14.3^{ef} \pm 1.62$	$0.167^{c} \pm 0.024$	$46.4^{a} \pm 3.04$
	F	$0.271^{ab} \pm 0.036$	$20.7^{cd} \pm 1.92$	$0.233^{ab} \pm 0.019$	$32.9^{c} \pm 2.96$
	E2+ F	$0.342^{ab} \pm 0.044$	$16.4^{def} \pm 2.15$	$0.186^{c} \pm 0.025$	$43.8^{a} \pm 4.59$
OVX	Vehicle	$0.263^{ab} \pm 0.029$	$27.3^{ab} \pm 2.33$	$0.254^{ab} \pm 0.024$	$22.4^{d} \pm 1.65$
	E2	$0.332^{ab} \pm 0.038$	$18.8^{de} \pm 1.66$	$0.216^{bc} \pm 0.014$	$40.7^{ab} \pm 3.03$
	F	$0.259^{b} \pm 0.044$	$30.9^{a} \pm 3.81$	$0.263^{a} \pm 0.026$	$23.4^{d} \pm 2.04$
	E2+ F	$0.290^{ab} \pm 0.052$	$23.7^{bc} \pm 2.40$	$0.238^{ab} \pm 0.020$	$34.3^{bc} \pm 3.13$

Table 2: Serum Cardiac profile and cardiac content of NOx

Conclusions

- Alteration of mitochondrial biogenesis and function is strongly related to decreased E2 after menopause.
- The demand for energy and excess mitochondrial-derived oxidative stress leads to myocardial injury, fibrosis and failure.
- A compensatory mechanism, via E2, ERα and ERβ, regulates nuclear and mitochondrial gene transcription.
- The current use of SERD, F, elaborates the role of ERα in E2-regulation of mitochondrial biogenesis and homeostasis via AMPK-related pathways, with molecular targets either completely or partially blocked by F indicating their contribution in controlling myocardial mitochondrial function.
- This outcome allows a deeper understanding of the role of ERs in heart function and that the responsiveness of ERα in cardiomyocytes is higher than ERβ, with a potential need to develop new drugs for cardiovascular diseases after menopause.