Biochemical and Biophysical Research Communications 480 (2016) 758–764

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Estrogen modulates exercise endurance along with mitochondrial uncoupling protein 3 downregulation in skeletal muscle of female mice

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ARTICLE INFO

Article history:
Received 21 October 2016
Accepted 28 October 2016
Available online 29 October 2016

Keywords:
Estrogen
Muscle
Endurance
Mitochondria
Ucp3

ABSTRACT

Estrogen is a hormone that regulates physiological processes and its dysregulation may relate to muscle disorders particularly in female, although the mechanism remains to be elucidated. We here show that estrogen deficiency repressed exercise endurance in female mice whereas the administration of estrogen to ovariectomized mice recovered it. Microarray analysis of mouse muscles showed that mitochondrial uncoupling protein 3 (UCP3) is upregulated by ovariectomy and downregulated by estrogen administration. Intriguingly, ectopic expression of constitutively active estrogen receptor α decreased UCP3 level and increased cellular ATP content in differentiated myoblastic C2C12 cells. Overall, the present study suggests that estrogen plays a critical role in the regulation of energy expenditure and exercise endurance in female.

1. Introduction

Estrogen is a sex steroid hormone that plays physiological roles in reproductive organs such as uterus, ovary and mammary gland as well as in diseases [1]. Estrogen also exerts physiological functions in non-reproductive organs/tissues such as bone, blood vessel, and brain. Indeed, estrogen withdrawal in postmenopausal women is assumed as risk factors for osteoporosis, arteriosclerosis, and Alzheimer's disease [2–4]. It is also hypothesized that estrogen exerts beneficial effects on skeletal muscle [5] since sarcopenia is frequently observed in postmenopausal women [6]. Several studies reported that women exhibit an accelerated decline in muscle strength around the time of menopause although men exhibit a gradual decrease in muscle strength across aging [7]. Besides, estrogen replacement therapy is reported to prevent muscle strength loss among peri- and post-menopausal women [8]. Furthermore, estrogen decline is assumed to be a causal factor in muscle mass loss since several lines of evidence imply that muscle mass is correlated with estrogen levels in women [9]. These findings imply a positive association between intensity of estrogen signaling and muscle strength/mass, although the precise mechanism remains to be clarified.

Some animal studies also suggest a link between estrogen signaling and muscle strength/mass [10–12]. It was reported that ovariectomy (OVX) in rats impair the recovery from atrophied muscle mass induced by hindlimb unloading [10]. Ovariectomized mice exhibited a significant decrease in muscle weight after muscle injury induced by cardiotoxin [11]. Moreover, ovariectomized rats supplemented with 17β-estradiol could be recovered from disuse muscle atrophy as quickly as control animals [12], suggesting that estrogen would be required for muscle regeneration. Nevertheless, it remains to be clarified whether estrogen has a beneficial effect on skeletal muscle, especially in exercise performance or endurance.

The biological function of estrogen is mediated through estrogen receptors (ERs), ERα and ERβ [1,13]. ERs are ligand-dependent transcription factors that regulate the expression of estrogen-responsive genes through binding to estrogen response elements (EREs) in the transcription-regulatory regions of the target genes. ERs are also expressed in skeletal muscles and assumed to regulate

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http://dx.doi.org/10.1016/j.bbrc.2016.10.129
0006-291X/

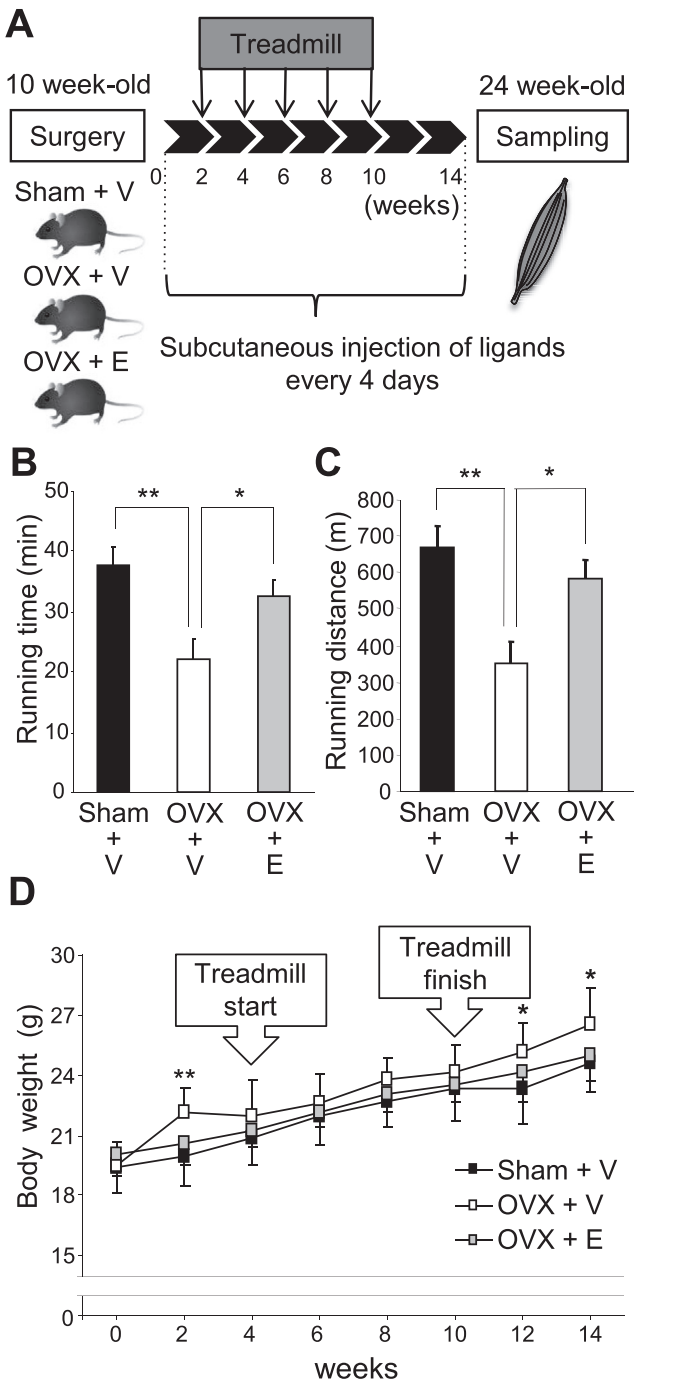

Fig. 1. Estrogen modulates exercise endurance in young female mice. (**A**) Summary of experimental design. Female C57BL/6J mice aged 10 week-old were underwent surgery. Mice treated with ovariectomy (OVX) were administered with estradiol benzoate (OVX + E group: $n = 10$) or vehicle (OVX + V group: $n = 10$) every 4 days. As a control group, sham-operated mice were administered with vehicle (Sham + V group: $n = 9$). Mice were exercised on a treadmill between 2 and 10 weeks after surgery and sacrificed at 24 week-old. (**B** and **C**) Mice were run on a treadmill with increasing speed to exhaustion. Maximal endurance running time (minutes)(B) and distance (meters)(C) were calculated at 10 weeks after surgery. Values are represented as means ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$ using Student's *t*-test. (**D**) Changes in absolute body weight after surgery. Data are expressed as means ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$ *versus* Sham + V group using Student's *t*-test.

metabolic pathways of glucose metabolism and insulin resistance [14]. A recent study of skeletal muscle-specific ERα knockout mice showed that ERα is required for glucose homeostasis, adiposity, and mitochondrial function in skeletal muscle of female [15].

In the present study, we explored the effects of estrogen on exercise performance and muscle activity in female mice. Animals were categorized into 3 groups: i) ovariectomized and vehicle treated (OVX + V), ii) ovariectomized and estradiol benzoate treated (OVX + E), and iii) sham operated and vehicle treated (Sham + V) groups. Treadmill test revealed that OVX + V mice significantly exhibit shorter running time and distance compared with Sham + V or OVX + E mice. We focused on uncoupling protein 3 (UCP3) expression in muscles as it was upregulated in OVX + V mice compared with Sham + V or OVX + E mice. Intriguingly, ectopic expression of constitutively active ERα also downregulated UCP3 levels in differentiated myoblastic C2C12 cells. These findings suggest that estrogen could play a critical role in the regulation of energy expenditure and exercise endurance in female.

2. Materials and methods

2.1. Mice and cyclic estrogen treatment

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Saitama Medical University. Female C57BL/6 mice were obtained from CREA Japan, Inc. at 8 weeks of age, maintained under specific pathogen-free conditions in temperature and humidity-controlled rooms (23 ± 2 °C, 50± 10%) with a 12 h light/dark cycle, and provided standard mouse chow and water *ad libitum*. Ten week-old mice were randomly divided into three groups with matched average body weight. One group was received sham surgery (Sham) and the other two groups were subjected to ovariectomy (OVX) by a dorsolateral approach under anesthesia. Three days after surgeries, cyclic estrogen or ethanol vehicle treatment was started. Namely, every 4 days, OVX mice were injected subcutaneously with 0.2 μg 17β-estradiol-3-benzoate (Sigma-Aldrich)(OVX + E group, $n = 10$) or control ethanol vehicle (OVX + V group, $n = 10$), respectively, in 100 μl corn oil (Nacalai tesque), and Sham-operated mice were administered with control ethanol vehicle in 100 μl corn oil (Sham + V group, $n = 9$). The body weight of the mice was measured once a week.

2.2. Exercise endurance test and grip strength test

Mice were exercised on a treadmill (MK-690S/RM, Muromachi) at 2, 4, 6, 8, and 10 weeks after surgeries. Prior to endurance exercise test, all mice were acclimatized to the treadmill running as described previously [16]. The exercise test regimen was 15 m min^{-1} for the first 10 min, followed by an increase of 2 m min^{-1} every 10-min intervals until exhaustion. Exhaustion was defined as a state when the mice were unable to avoid repetitive electrical shocks and stayed more than 14 s in 60 s on the electrical grid. Time to exhaustion (minutes) and total running distance (meters) were evaluated. Forelimb strength of mice was measured at 10 weeks after surgery as described [17]. Five tests were performed for each mouse and the maximum data was recorded. At 14 weeks after surgery, mice were sacrificed by anesthesia overdose and tissues were dissected.

2.3. Microarray analysis

Total RNA was isolated from the soleus muscle using ISOGEN (Nippon Gene) and subjected to microarray analysis using Affymetrix GeneChip (Mouse Gene 1.0 ST Array). A global analysis of gene expression and differentially expressed genes in pathways and clusters of functionally related genes were performed using the DAVID Functional Annotation Clustering Tool (http://david.abcc.ncifcrf.gov/summary.jsp).