Biochemical and Biophysical Research Communications 392 (2010) 1–3

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Loss of ovarian function in mice results in abrogated skeletal muscle PPARδ and FoxO1-mediated gene expression

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ARTICLE INFO

Article history:
Received 28 September 2009
Available online 20 October 2009

Keywords:
Estrogen
Menopause
Atrogin-1/MAFbx
MuRF-1
Musclin
Myogenin

ABSTRACT

Menopause, the age-related loss of ovarian hormone production, promotes increased adiposity and associated metabolic pathology, but molecular mechanisms remain unclear. We previously reported that estrogen increases skeletal muscle PPARδ expression *in vivo*, and transgenic mice overexpressing muscle-specific PPARδ are reportedly protected from diet-induced obesity. We thus hypothesized that obesity observed in ovariectomized mice, a model of menopause, may result in part from abrogated expression of muscle PPARδ and/or downstream mediators such as FoxO1. To test this hypothesis, we ovariectomized (OVX) or sham-ovariectomized (SHM) 10-week old female C57Bl/6J mice, and subsequently harvested quadriceps muscles 12 weeks later for gene expression studies. Compared to SHM, muscle from OVX mice displayed significantly decreased expression of PPARδ (3.4-fold), FoxO1 (4.5-fold), PDK-4 (2.3-fold), and UCP-2 (1.8-fold). Consistent with studies indicating PPARδ and FoxO1 regulate muscle fiber type, we observed dramatic OVX-specific decreases in slow isoforms of the contractile proteins myosin light chain (11.1-fold) and troponin C (11.8-fold). In addition, muscles from OVX mice expressed 57% less myogenin (drives type I fiber formation), 2-fold more MyoD (drives type II fiber formation), and 1.6-fold less musclin (produced exclusively by type II fibers) than SHM, collectively suggesting a shift towards less type I oxidative fibers. Finally, and consistent with changes in PPARδ and FoxO1 activity, we observed decreased expression of atrogin-1 (2.3-fold) and MuRF-1 (1.9-fold) in OVX mice. In conclusion, muscles from ovariectomized mice display decreased PPARδ and FoxO1 expression, abrogated expression of downstream targets involved in lipid and protein metabolism, and gene expression profiles indicating less type I oxidative fibers.

Introduction

Menopause promotes increased visceral adiposity and type 2 diabetes [1], but mechanisms remain unclear. Skeletal muscle, responsible for greater than 70% of whole body insulin stimulated glucose uptake, expresses estrogen receptors and is thus a likely target of estrogenic actions.

Skeletal muscle is comprised of both type I "slow" oxidative fibers and type II "fast" glycolytic fibers. Peroxisome proliferator-activated receptor-δ (PPARδ) is a transcription factor that potently drives skeletal muscle fat oxidation [2] and type I slow oxidative fiber development [3]. Importantly, obesity is inversely associated with the proportion of oxidative slow-twitch fibers [4]. We have previously shown that exogenous estrogen increases skeletal muscle PPARδ expression in ovariectomized mice [5], suggesting ovariectomy promotes obesity in part by decreasing skeletal muscle PPARδ expression. We demonstrate here that ovariectomized mice, as a model of menopause, display abrogated PPARδ and FoxO1 related gene expression in skeletal muscle, with evidence of a shift towards less type I oxidative fibers. These gene expression changes could help explain the decreased energy expenditure and increased adiposity we previously observed in this model [6].

Materials and methods

Animals. Experiments were conducted in a viral pathogen–free facility at the Jean Mayer-U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University in accordance with institutional animal care and use committee guidelines. SHM-ovariectomized (SHM, $n = 10$) and ovariectomized (OVX, $n = 11$) C57Bl/6J mice were purchased from Charles River, with operations performed at 10 weeks of age. Animals were individually housed with 12-h light/dark cycles and given free access to water and food (phytoestrogen free chow, Harlan Teklad).

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doi:10.1016/j.bbrc.2009.10.072

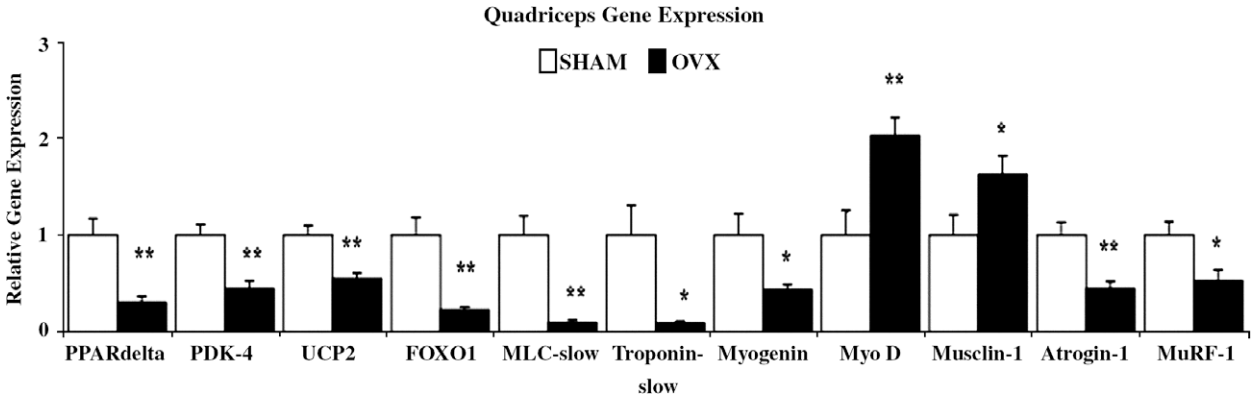

Fig. 1. OVX mice have altered PPARδ and FoxO1-mediated oxidative gene expression. Real-time PCR was used to determine expression of genes (relative to the endogenous control cyclophilin B) in quadriceps from sham-ovariectomized (SHAM, white bars) and ovariectomized (OVX, black bars) mice. MLC = myosin light chain. Error bars indicate SE of the mean. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Quantitative Polymerase Chain Reaction (QPCR). After 12 weeks, mice were euthanized, and quadriceps muscles dissected and immediately snap frozen in liquid nitrogen and stored at −80 °C. RNA was isolated using commercial spin kits (Ambion, Austin, TX) according to manufacturer's instructions. RNA was quantified and checked for purity using the Nanodrop spectrophotometer (Nanodrop 1000, Wilmington, DE). cDNA was generated from 1 μg of RNA using AMV Reverse Transcriptase (Promega, Madison, WI), and QPCR performed using SYBR Green (Applied Biosystems 7300, Foster City, CA). Fold changes were calculated as $2^{-\Delta\Delta CT}$ compared to the endogenous control gene cyclophilin B. Primer sequences available upon request.

Statistical analyses. Comparisons were made using student's *t*-tests and data presented are means + SEM. Significance was defined as $p < 0.05$.

Results

Quadriceps from OVX mice displayed significantly decreased PPARδ expression (3.4-fold). In addition, expression of downstream targets of PPARδ that mediate lipid oxidation were decreased in muscle from OVX mice. These genes include pyruvate deyhdrogenase kinase-4 (PDK-4; 2.3-fold, $p < 0.001$), uncoupling protein-2 (UCP-2; 1.8-fold, $p < 0.001$) and the forkhead box transcription factor family member FoxO1 (4.5-fold, $p < 0.001$) (Fig. 1).

In addition to altering fuel utilization, PPARδ and FoxO1 promote type I (slow oxidative) fiber development by increasing the expression of slow isoforms of contractile proteins (i.e. troponins, myosins) [7]. OVX resulted in dramatically decreased expression of the myosin light chain slow isoform (11.1-fold, $p < 0.001$), and troponin C slow isoform (11.8-fold, $p < 0.05$). Moreover, compared to SHM mice, quadriceps from OVX mice expressed 50% less myogenin ($p < 0.05$) and twice as much myoD ($p < 0.01$). These changes are consistent with a fiber type shift, as myogenin specifically promotes the development of type I oxidative fibers [8], while myoD promotes the development of type II glycolytic fibers [9]. Finally, the expression of musclin, which is negatively regulated by FoxO1 [10] and made exclusively by type II fibers [11], was increased 1.6-fold ($p < 0.05$) in muscle from OVX mice. These data, presented in Fig. 1, collectively suggest that OVX in mice results in a fiber type switch toward a less type I oxidative muscle.

PPARδ [12] and FoxO1 [13] also regulate expression of atrophy gene-1/muscle atrophy F-box (Atrogin-1/MAFbx) and muscle ring-finger protein 1 (MuRF-1), which are ubiquitin ligases involved in muscle atrophy. OVX muscle displayed 2.3-fold less Atrogin-1/MAFbx ($p < 0.001$) and 1.9-fold less MuRF-1 ($p < 0.05$) expression (Fig. 1). Decreases in these genes are consistent with changes in PPARδ and FoxO1 expression, and suggest a novel role for ovarian hormones in protein metabolism.

Discussion

Estrogen receptor expression [14] makes muscle a likely target tissue for ovarian hormone action, but studies investigating the impact of ovarian hormones on skeletal muscle are limited. We show here that PPARδ and downstream mediators of oxidative metabolism, FoxO1, PDK-4 and UCP-2, are decreased in muscle from OVX mice. Moreover, expression of slow isoforms of contractile proteins were decreased in quadriceps from OVX and SHM mice, consistent with other models demonstrating fiber-type switching via ablated PPARδ [15] or FoxO1 [7]. Also, similar to changes reported with FoxO1-ablation induced fiber-type switching in skeletal muscle [7], OVX mice displayed decreased myogenin and increased myoD [9] expression. Finally, and again consistent with less type I oxidative fibers, expression of musclin, which is produced exclusively by type II fibers, was increased in OVX mouse muscle. Collectively, these data (summarized in Fig. 2) suggest that ovariectomy-induced changes in PPARδ and FoxO1 may lead to an abrogated capacity for fatty acid oxidation and detrimental shifts in fiber type that are likely to contribute to ovariectomy-induced adiposity [6].

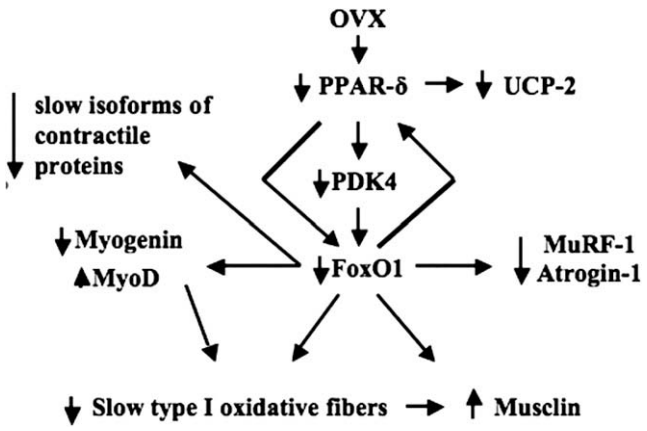

Fig. 2. Summary of OVX-induced gene expression changes in mouse quadriceps: OVX decreases PPARδ expression, leading to decreased UCP-2, PDK-4 and FoxO1 expression. Attenuated FoxO1 expression is concomitant with decreased myogenin and increased myoD expression, promoting less type I fiber development. Consistent with a fiber type shift, as only type II fibers produce musclin, OVX mice display increased musclin expression. Expression of PPARδ and FoxO1 downstream targets involved in protein metabolism (e.g. MuRF-1, atrogin-1) are similarly decreased.

PPARδ promotes catabolism of not only fatty acids, but also proteins, the latter mediated via upregulated atrogin-1/MAFbx and MuRF-1 expression (muscle specific ubiquitin ligases involved in atrophy). Consistent with the observed decreased PPARδ expression, we found atrogin-1 and MuRF-1 expression to be attenuated in skeletal muscle from ovariectomized mice. These changes are consistent with observed links between estrogen status and protein turnover [16], as atrogin-1 and MuRF-1 have been recently implicated as important regulators of healthy protein turnover [17].

We conclude that ovariectomy alters PPARδ and FoxO1 related gene expression in murine skeletal muscle. These observations provide a potential explanation for the observed increase in adiposity and co-morbidities with menopause. Future detailed studies are warranted.

Acknowledgments

The authors would like to thank Dr. Richard Karas and Dr. Jennifer Sacheck for their thoughtful insight during manuscript preparation. This work was supported by U.S. Department of Agricultural Research Service under cooperating agreement no. 58-1950-7-707 and National Institutes of Health Grants T32DK062032, DK-50647, and DK074979. A.S.G. is a recipient of an endowed chair from the Robert C. and Veronica Atkins Foundation.

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