Influence of oestrogen on satellite cells and myonuclear domain size in skeletal muscles following resistance exercise

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Abstract

Background Oestrogen deficiency reduces skeletal muscle mass and force generation in postmenopausal women. Muscle mass is maintained by satellite cells, which are regulated by oestrogen. Although oestrogen therapy enhances muscle hypertrophy induced by resistance training in postmenopausal women, the molecular mechanism is unclear.

Methods Adult female rats (10 weeks old) were divided into six groups: sham sedentary (Sham-Sed), sham climbing training (Sham-CT), ovariectomy sedentary (OVX-Sed), ovariectomy climbing training (OVX-CT), ovariectomy plus oestrogen treatment sedentary (OVX+E-Sed), and ovariectomy plus oestrogen treatment climbing training (OVX+E-CT). At 8 weeks after ovariectomy, rats in the training group were trained (one session every 3 days for 8 weeks) to climb a ladder while bearing a load. Oestrogen treatment involved subcutaneous insertion of a 17β-oestradiol pellet. After 8 weeks, the flexor hallucis longus muscle was collected and analysed.

Results Following climbing training, the flexor hallucis longus muscle mass and muscle-to-body weight ratios were dramatically increased by training (main effect of training, \(P < 0.01\)); the OVX+E-CT group showed the highest values (main effect of group, \(P < 0.01\)). The cross-sectional area of all muscle fibre types was increased by training (main effect of training, \(P < 0.01\)). Particularly, the cross-sectional area of MHC IIa in the OVX+E-CT group was significantly larger than that in the Sham-CT and OVX-CT groups. Satellite cell numbers were increased in all training groups (main effect of training, \(P < 0.05\)), and the myonuclear number was increased by training (main effect of training, \(P < 0.01\)), but there was no main group effect. The myonuclear domain size of all muscle fibre types and MHC IIa was increased in all training groups (main effect of training, \(P < 0.01\)) and showed a main group effect (\(P < 0.01\)). The myonuclear domain sizes of all muscle fibre types and MHC IIa in the OVX+E-CT group were significantly larger than those in the Sham-CT and OVX-CT groups. The total RNA contents revealed main effects of training and the group (main effect of group, \(P < 0.01\)); the OVX+E-CT group showed the highest contents (main effect of group, \(P < 0.01\)). The mRNA and protein levels of rpS6 were increased in the OVX+E-Sed and CT groups (main effects of group, \(P < 0.05\)). Particularly, the 28S ribosomal RNA content in OVX+E-Sed group was significantly higher than that in the OVX-Sed group.

Conclusions Oestrogen enhanced the resistance training-induced increase in myonuclear domain size but did not affect satellite cells and ribosome biogenesis.

Keywords Menopause; Oestradiol; Resistance training; Satellite cell; Myonuclear domain size; Ribosome biogenesis

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Introduction

The progressive loss of muscle mass, strength, and physical function that occurs with aging is known as sarcopenia. Postmenopausal women have less lean mass, lower physical activity levels, and lower muscle quality compared with younger women, all of which contribute to decreased physical function. A lack of oestrogen after menopause may crucially contribute to reduced muscle quality in older women. Muscle mass is maintained by satellite cells, and previous studies demonstrated that oestrogen deficiency impairs satellite cell maintenance and function and the cells fail to regenerate effectively after muscle injury. Furthermore, oestrogen affects skeletal muscle regeneration by regulating downbell running-induced activation and proliferation of satellite cells. In contrast, satellite cells contribute to resistance training-induced muscle hypertrophy. Satellite cell activity, ribosomal function (translational efficiency) and ribosome biogenesis (translational capacity), and transcriptional regulation are involved in the molecular regulation of muscle hypertrophy induced by resistance training. Satellite cells fuse with muscle fibres during muscle hypertrophy and increase the number of myonuclear. MyoD and myogenin expression is increased by activated satellite cells and initiates proliferation and myogenic differentiation. Additionally, ribosomal function critically contributes to resistance training-induced muscle hypertrophy. Particularly, ribosome biogenesis augments the myonuclear domain size in hypertrophic muscle fibres. Taken together, satellite cell-dependent muscle hypertrophy leads to myonuclei addition, whereas satellite cell-independent muscle hypertrophy increases the myonuclear domain size. Dam et al. demonstrated that oestrogen therapy enhances muscle hypertrophy induced by resistance training in postmenopausal women; however, the molecular regulation of oestrogen in this process remains unclear.

This study was conducted to investigate the molecular mechanism of oestrogen regulation of resistance training-induced muscle hypertrophy in an ovariectomy and ladder climbing training animal model. We evaluated the effect of oestrogen on the cross-sectional area (CSA), muscle fibre myosin heavy chain (MHC) type, satellite cells, myonuclear domain size, and ribosome biogenesis following ladder climbing training.

Methods

Experimental animals

Thirty-six female F344 rats (8 weeks old) were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and housed under controlled environmental conditions (23 ± 1°C, 55 ± 5% relative humidity) with a 12-h light/dark cycle, ad libitum access to water, and a standard laboratory diet. The animals were randomly assigned to one of six experimental groups: sham sedentary (Sham-Sed), sham with climbing training (Sham-CT), ovariectomy and sedentary (OVX-Sed), ovariectomy with climbing training (OVX-CT), ovariectomy with oestrogen treatment and sedentary (OVX+E-Sed), or ovariectomy with oestrogen treatment and climbing training (OVX+E-CT). All animal experiments were conducted in compliance with the ethical requirements of the Animal Committee of Juntendo University (H29-01).

Ovariectomy and oestrogen treatment

At 10 weeks of age, the OVX and OVX+E groups were subjected to ovariectomy by dorsally removing the bilateral ovaries. Sham group rats were subjected to sham operation. Rats in the oestrogen treatment group were subcutaneously implanted with a 17β-oestradiol pellet (0.25 mg/pellet) (E-121; Innovative Research of America, Sarasota, FL, USA) at 8 weeks after ovariectomy and were treated for 8 weeks.

Ladder climbing training

The climbing training protocol was adapted from Hornberger and Farrar. Rats in the climbing training group were trained to climb a 1-m-long ladder with rungs that were 2 cm apart and inclined at 85°, with one training session every 3 days for a total of 20 training sessions over 8 weeks. Before the first training session, the rats were acclimated to ladder climbing without loading for 3 days. The first training session was performed at 8 weeks after surgery. The initial carrying loads were 50%, 75%, 90%, and 100% of the rat’s body weight, and a carrying load of 30 g was added for each climb repetition for up to 10 repetitions in the first training session. The load was progressively reduced when the rats climbed 10 repetitions because of the heavy load. The maximal carrying capacity of this training session was considered as the highest load that the rat carried while successfully climbing to the top of the ladder. In training sessions 2–20, the initial carrying loads were 50%, 75%, 90%, and 100% of the maximal carrying capacity of the last training session, and the load was progressively increased by 30 g.

Muscle sampling

At 48 h after the last training session, the hindlimb skeletal muscles (soleus, plantaris, and flexor hallucis longus [FHL] muscles) from both legs were carefully dissected, weighed, and frozen in liquid nitrogen for biochemical analysis or in isopentane cooled by liquid nitrogen for immunohistochemi-
cal analysis. The muscle samples were stored at −80°C until analysis.

**Quantification of plasma oestradiol**

The plasma was collected by centrifugation at 3000 rpm for 10 min at 4°C. The internal standards $^{13}$C$_4$E$_2$ (E$_2$-$^{13}$C$_4$) were added to the plasma that had been diluted with water. The sample and standards were mixed with methyl tert-butyl ether, and the methyl tert-butyl ether layer was transferred and evaporated to dryness. The extract was dissolved in methanol and diluted with distilled water. The sample was applied to a successively conditioned Oasis MAX cartridge. After the cartridge was washed with 1% acetic acid solution, 30% acetonitrile solution, 1 M sodium hydroxide solution, methanol, 1% acetic acid solution, and methanol/distilled water/pyridine (60:40:1, v/v/v), E$_2$ was eluted with methanol/distilled water/pyridine (90:10:1, v/v/v). After evaporation, the residue was reacted with pentafluoropyridine, triethylamine, and acetonitrile at 25°C for 20 min and evaporated to dryness. For further derivatization, the residue was reacted with 2-methyl-6-nitrobenzoic anhydride, fusaric acid, 4-dimethylaminopyridine, acetonitrile, and triethylamine at room temperature for 30 min and then purified using an InertSep SI cartridge (GL Sciences Inc., Tokyo, Japan). After purification, the residue was evaporated to dryness and dissolved in acetonitrile/distilled water (80:20, v/v). The solution was subjected to liquid chromatography–tandem mass spectrometry. The limit of quantification of E$_2$ was 0.005 pg/tube.

**Immunohistochemistry**

To determine the CSA and analyse individual muscle fibres, MHC typing was carried out using the midbelly region of the FHL muscles. Frozen cross-sections (10 μm) were stained with mouse monoclonal antibodies against MHC I, IIa, and IIx and rabbit polyclonal antibodies against laminin. Briefly, the muscle sections were fixed in phosphate-buffered saline (PBS) containing 4% paraformaldehyde and permeabilized with PBS containing 1% Triton X-100. The sections were blocked with 10% normal goat serum (NGS) in PBS at 25°C for 1 h and then incubated with primary antibodies at room temperature for 1 h. The primary antibodies anti-MHC I (clone BA-F8) (1:100), anti-MHC IIa (clone SC-71) (1:100), anti-MHC IIx (6H-1) (1:100) (Developmental Studies Hybridoma Bank, Coralville, IA, USA), and anti-laminin (Sigma-Aldrich, St. Louis, MO, USA) (1:100) were diluted in PBS containing 5% NGS. To visualize the satellite cells and analyse the myonuclear domain size, the cells were incubated with the following primary antibodies diluted in PBS containing 5% NGS at 4°C overnight: anti-Pax7 (1:5), anti-MHC IIx (6H-1) (Developmental Studies Hybridoma Bank) (1:100), and anti-laminin (Sigma-Aldrich) (1:100). After washing with PBS, the sections were incubated for 1 h at 25°C with secondary antibodies (goat anti-mouse IgG [Invitrogen, Carlsbad, CA, USA] for BA-F8 and SC-71, goat anti-mouse IgM [Invitrogen] for 6H-1, and goat anti-rabbit IgG [Invitrogen] for laminin). The secondary antibodies were diluted to 1:500 in PBS containing 5% NGS. Images of the stained sections were obtained under a fluorescence microscope (BZ-X800; Keyence, Osaka, Japan). The CSA of approximately 200 fibres/muscle sections was calculated using Keyence analyser software. To analyse the muscle fibre type composition, the number of muscle fibres was counted for each muscle fibre type from approximately 500–600 fibres.

**Protein extraction**

Flexor hallucis longus muscles were formed into a powder by freezing in liquid nitrogen and then homogenized in T-PER Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA, USA) containing protease (Thermo Fisher Scientific) and phosphatase inhibitors (Roche Diagnostics GmbH, Mannheim, Germany). The protein concentration was measured using BCA protein assay reagent according to the manufacturer’s instructions (Thermo Fisher Scientific).

**Western blot analysis**

The muscle protein extracts (20 μg) were separated by electrophoresis using 12% sodium dodecyl sulfate polyacrylamide gels and electro-blotted onto polyvinylidene fluoride membranes. The blots were stained with Ponceau S to verify the equal loading of proteins. The membranes were blocked with 5% skim milk in Tris-buffered saline/Tween 20 (TBST) for 1 h at 25°C and then incubated with anti-total ribosomal protein S6 (rpS6) primary antibody (cat. no. 2217) (Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. The blots were washed three times with TBST and incubated with a 1:5000 dilution of horseradish peroxidase-conjugated secondary antibody (ab6721, Abcam, Cambridge, UK) for 1 h at 25°C. After washing the blots three times with TBST, they were developed using an enhanced chemiluminescence substrate (Amersham, Buckinghamshire, UK). Band intensities were quantified using Image Lab v.5.2.1 software (Bio-Rad Laboratories, Hercules, CA, USA).

**RNA isolation**

The FHL muscle powder was homogenized in 1 mL ISOGEN (FUJIFILM Wako Pure Chemical, Osaka, Japan); chloroform was added to the homogenate, which was shaken vigorously for 15 s and then left standing at room temperature for...
3 min. The mixture was centrifuged at 15 000×g for 15 min at 4°C, and the aqueous phase was transferred to a fresh tube. Isopropanol was added to this aqueous solution to precipitate the RNA. After incubation at 25°C for 10 min, the tube was centrifuged at 15 000×g for 15 min at 4°C. The recovered RNA pellet was washed with 70% ethanol, centrifuged at 15 000×g for 10 min at 4°C, and air-dried for 5 min. The RNA was dissolved in RNase-free water and treated with DNase (Invitrogen) to remove residual genomic DNA. RNA concentrations were determined by measuring the absorbance at 260 nm.

Quantification of ribosomal RNA

The total RNA solution was adjusted to 1 μL RNA solution per 1 mg muscle according to the initial muscle powder. An RNA solution equivalent to 125 μg muscle tissue was stained with fluorescent dye (GRR-1000GR Red; Bio-Craft, Tokyo, Japan) and electrophoresed on a 1% agarose gel in Tris-borate-EDTA buffer. The bands in the gel were viewed under ultraviolet light. The band intensities of 18S ribosomal RNA (rRNA) and 28S rRNA were quantified using Image Lab v5.2.1 (Bio-Rad Laboratories).

Quantitative polymerase chain reaction

The cDNA was synthesized from RNA using SuperScript III reverse transcriptase (cat. no. 18080-044) (Invitrogen) according to the manufacturer’s instructions. Real-time PCR was performed using the QuantStudio™ 3 System (Applied Biosystems, Foster City, CA, USA) and THUNDERBIRD SYBR® qPCR Mix (cat. no. QPS201) (Toyobo, Osaka, Japan) according to the manufacturer’s instructions. The amplification protocol consisted of denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. A standard curve was designed to quantitatively analyse mRNA expression levels; an aliquot of each experimental sample was used to generate the standard curve. The following primers were used: rpS6 forward 5’-AGAGGAAGCGCAAGTCTGTC-3’, rpS6 reverse 5’-CGACGAGGCCAGTGGTATC-3’, and GAPDH forward 5’-TGAACGGGAAGCTCAGTGC-3’, GAPDH reverse 5’-TCCACACACGTGGTATC-3’. GAPDH was used for normalization.

Statistical analysis

All data are expressed as the mean ± standard deviation. Statistical analysis was performed using two-way analysis of variance followed by Tukey’s post-hoc test with GraphPad Prism 5 software (GraphPad, Inc., San Diego, CA, USA). Statistical significance was set at P < 0.05.

Results

Body weight, ovarian adipose tissue, uterus, soleus, and plantaris masses and plasma oestradiol. The body weight and ovarian adipose tissue mass of the rats were increased by ovariectomy surgery (main effect of group, body weight, P < 0.01, ovarian adipose, P < 0.05) and decreased by training (main effect of training, P < 0.01). The uterine mass was determined to confirm successful ovariectomy and oestrogen treatment. The uterine mass was decreased by ovariectomy surgery and increased by oestrogen treatment (main effect of group, P < 0.01). Furthermore, the plasma oestradiol concentration was also decreased by ovariectomy surgery and increased by oestrogen treatment (main effect of group, P < 0.01). There was no main effect of training on the soleus and plantaris masses (Table 1).

|                  | Body weight (g) | Ovarian adipose tissue (mg) | Uterus (mg) | Soleus (mg) | Plantaris (mg) | Oestradiol (pg/mL) |
|------------------|----------------|---------------------------|-------------|-------------|----------------|-------------------|
| Sham-Sed         | 169 ± 7.5      | 4294 ± 1014.6             | 451 ± 92.1  | 70.3 ± 4.9  | 185 ± 13.3      | 5.02 ± 5.91       |
| Sham-CT          | 162 ± 7.3      | 2784 ± 777.7              | 329 ± 82.6  | 67.8 ± 6.1  | 181 ± 12.7      | 7.30 ± 4.37       |
| OVX-Sed          | 188 ± 6.3      | 5813 ± 1758.1             | 135 ± 60.9  | 70.3 ± 3.7  | 193 ± 11.6      | 0.39 ± 0.23       |
| OVX-CT           | 172 ± 5.7      | 3120 ± 816.3              | 154 ± 89.3  | 69.4 ± 4.9  | 192 ± 9.7       | 0.71 ± 0.87       |
| OVX+E-Sed        | 174 ± 10.9     | 3749 ± 1384.4             | 636 ± 45.9  | 74.1 ± 10.6 | 196 ± 13.4      | 320 ± 196         |
| OVX+E-CT         | 167 ± 7.6      | 2331 ± 775.1              | 648 ± 86.6  | 70.5 ± 6.2  | 198 ± 10.3      | 180 ± 110         |

Sham-Sed, sham sedentary; Sham-CT, sham climbing training; OVX-Sed, ovariectomy sedentary; OVX-CT, ovariectomy climbing training; OVX+E-Sed, ovariectomy plus oestrogen treatment sedentary; OVX+E-CT, ovariectomy plus oestrogen treatment climbing training. (*, **) Main effect of training. (#, ##) Main effect of group. * P < 0.05, ** P < 0.01, # P < 0.05, ## P < 0.01.
**FHL muscle mass, protein content, and CSA**

In a previous study, the FHL muscle responded markedly to muscle hypertrophy after climbing training. The FHL muscle mass and muscle-to-body weight ratios were dramatically increased by training (main effect of training, $P < 0.01$). The FHL muscle mass and muscle-to-body weight ratios in the OVX+ECT group were higher than those in the other groups (main effect of group, $P < 0.01$) (Figure 1A,B). The protein content was increased by training (main effect of training, $P < 0.01$) and was highest in the OVX+E-CT group (main effect of group, $P < 0.01$) (Figure 1C). The main effects of training and group were evident for all muscle fibre type CSAs of the FHL muscle and trended towards a significant interaction ($P = 0.09$) (Figure 2B). The CSA of the MHC I muscle fibre showed a main effect of training, but no main effect of group (Figure 2C). The CSA of the MHC IIa muscle fibre was increased by training (main effect of training, $P < 0.01$). Notably, the CSA of the MHC IIb muscle fibre was significantly larger in the OVX+E-CT group than in the Sham-CT and OVX-CT groups (Figure 2D). The CSA of the MHC IIx/IIb muscle fibre was increased by training (main effect of training, $P < 0.01$), and that of the MHC IIx/IIb muscle fibre of OVX+E-CT group was largest among all groups (main effect of group, $P < 0.01$) (Figure 2E).

**Muscle fibre type composition of FHL muscle**

The FHL muscle contains MHC (3.5% MHC I, 22.8% MHC IIa, 41.7% MHC IIx, and 31.9% MHC IIb). All training groups showed a higher proportion of MHC IIa and IIx compared with the sedentary groups. Specifically, MHC IIb was not detected in the training groups (Table 2).

**Satellite cell and myonuclear number of FHL muscle**

The number of satellite cells increased in all training groups (main effect of training, $P < 0.05$) but there was no main effect of the group (Figure 3C). The myonuclear number was increased by training (main effect of training, $P < 0.01$) for all muscle fibre types, MHC IIa, and MHC IIx/IIb but there was no main effect of the group (Figure 4A,C,D). There were no main effects of training or the group on the myonuclear number of MHC I (Figure 4B).

**Myonuclear domain size of FHL muscle**

The myonuclear domain size of all muscle fibre types was increased in all training groups (main effect of training,
$P < 0.01$) and showed a main effect of the group. Particularly, the myonuclear domain size of all muscle fibre types in the OVX+E-CT group was significantly larger than those in the Sham-CT and OVX-CT groups (Figure 5A). The myonuclear domain size of MHC I revealed the main effects of the training and group but the interaction was not significant (Figure 5B). However, there was a significant interaction for the myonuclear domain size of MHC IIa, and the value in the

Figure 2 Effects of climbing training and oestrogen on the cross-sectional area of flexor hallucis longus (FHL) muscle. (A) Representative immunohistochemical staining of MHC. The reactivities of the major fibre types, including type I (red), type IIa (blue), and type IIx (green), are shown for FHL muscles. The cross-sectional area of FHL muscle. (B) All muscle fibre type, (C) MHC I, (D) MHC IIa, (E) MHC IIx/IIb. Values are presented as the mean ± SD ($n = 6$), *$P < 0.05$, scale bar: 100 μm. Sham-Sed, sham sedentary; Sham-CT, sham climbing training; OVX-Sed, ovariectomy sedentary; OVX-CT, ovariectomy climbing training; OVX+E-Sed, ovariectomy plus oestrogen treatment sedentary; OVX+E-CT, ovariectomy plus oestrogen treatment climbing training.
OVX+E-CT group was significantly larger than that in the Sham-CT and OVX-CT groups (Figure 5C). The myonuclear domain size of MHC IIx/IIb showed main effects for training and the group and a trend towards a significant interaction ($P = 0.06$), with the largest value observed in the OVX+E-CT group (Figure 5D).

**Total RNA and ribosome biogenesis of FHL muscle**

The total RNA content indicated the involvement of ribosome biogenesis during muscle hypertrophy. The total RNA content revealed the main effects of the training and group ($P < 0.01$), with the highest RNA content observed in the OVX+E-CT group (main effect of group, $P < 0.01$) (Figure 6A). The 40S ribosomal subunit rpS6 is an indicator of ribosome biogenesis. The mRNA level of rpS6 revealed the main effects of the group ($P < 0.01$), with the OVX+E-CT group showing the highest mRNA level of rpS6 (Figure 6B). The total protein level of rpS6 showed the main effects of the group ($P < 0.01$) (Figure 6C). Neither the 18S + 28S rRNA content nor 18S rRNA content were significantly different among all groups. The 28S rRNA content showed a significant interaction ($P < 0.05$) and trend towards a significant main effect.

### Table 2  Muscle fibre type distribution in flexor hallucis longus muscle

|                | MHC I | MHC IIa | MHC IIx | MHC IIb |
|----------------|-------|---------|---------|---------|
| Sham-Sed       | 3.5 ± 1.1 | 22.8 ± 1.7 | 41.4 ± 3.8 | 31.9 ± 2.3 |
| Sham-CT        | 4.6 ± 2.0 | 28.0 ± 3.8 | 67.4 ± 2.5 | 0.0       |
| OVX-Sed        | 3.2 ± 1.3 | 22.7 ± 1.8 | 41.0 ± 5.8 | 33.0 ± 4.2 |
| OVX-CT         | 4.2 ± 1.1 | 28.4 ± 7.1 | 67.4 ± 7.0 | 0.0       |
| OVX+E-Sed      | 2.8 ± 1.5 | 27.3 ± 7.3 | 43.7 ± 8.5 | 26.3 ± 11.4 |
| OVX+E-CT       | 4.2 ± 1.3 | 35.8 ± 2.9 | 60.0 ± 3.7 | 0.0       |

Sham-Sed, sham sedentary; Sham-CT, sham climbing training; OVX-Sed, ovariectomy sedentary; OVX-CT, ovariectomy climbing training; OVX+E-Sed, ovariectomy plus oestrogen treatment sedentary; OVX+E-CT, ovariectomy plus oestrogen treatment climbing training.

**Figure 3** Effects of climbing training and oestrogen on the number of satellite cell of flexor hallucis longus (FHL) muscle. The representative immunohistochemical staining of satellite cell (A) The satellite cells with Pax7$^+$ (green), and laminin (white). (B) The satellite cell with Pax7$^+$ (green), myosin heavy chain (MHC) IIx (red), myonuclear (blue), and laminin (white). (C) Effects of climbing training and oestrogen on the number of satellite cell of flexor hallucis longus (FHL) muscle. Values are presented as mean ± SD ($n = 6$); *$P < 0.05$, scale bar: 100 μm. Sham-Sed, sham sedentary; Sham-CT, sham climbing training; OVX-Sed, ovariectomy sedentary; OVX-CT, ovariectomy climbing training; OVX+E-Sed, ovariectomy plus oestrogen treatment sedentary; OVX+E-CT, ovariectomy plus oestrogen treatment climbing training.
for training ($P = 0.06$). Particularly, the OVX+E-Sed group showed a significantly higher 28S rRNA content compared with that in the OVX-Sed group (Figure 6D).

**Discussion**

We demonstrated that oestrogen enhanced resistance training-induced muscle hypertrophy by dramatically augmenting MHC IIa muscle fibre hypertrophy. Our data suggest that oestrogen influences ribosome biogenesis, RNA and protein contents, and myonuclear domain size to regulate resistance training-induced muscle hypertrophy at the molecular levels but does not affect resistance training-induced increases in satellite cells.

Successful ovariectomy and oestradiol treatment were confirmed by measuring the uterine mass and bodyweight of the rats. A reduced uterine weight and increased body weight are typical phenotypes following ovariectomy. Thus, ovariectomy was considered as successful for preparing the model in the present study. Hornberger and Farrar reported a 23% increase in the mass of FHL muscle in male rats following 8 weeks of ladder-climbing training. Lee et al. showed an 8.9% increase in the CSA of the FHL muscle in female rats following 8 weeks of ladder-climbing training. Our data revealed a 15% increase in mass and 14% increase in CSA of the FHL muscle in female Sham-CT rats compared with Sham-Sed rats. Muscle hypertrophy induced by resistance training was observed in the rats.

Satellite cells were increased following downhill running and were enhanced by oestrogen in ovariectomy rats, whereas the satellite cell number and muscle regeneration were impaired in ovariectomy rats. Thus, oestrogen has important effects on satellite cells after muscle injury. Our data showed that satellite cells increased following climbing training; however, this increase was not influenced by oestrogen. Climbing training may disrupt muscle fibres in a manner that differs from that in downhill running-induced muscle injuries.

Another reason for the lack of increase in satellite cell numbers may be the sampling time of muscle following oestrogen supplementation. Enns and Tiidus demonstrated that the number of satellite cells in ovariectomy rats administered oestrogen was increased at 72 h following a single bout of downhill running. The sampling time (48 h post-exercise) of muscle in the present study may be too early to determine the satellite cell numbers. Muscle sampling can also be performed during resistance training. Damas et al. reported a significant increase in the number of satellite cells after 10 weeks of resistance training; however, the number of satellite cells was not increased at 48 h following a single bout of resistance exercise after 10 weeks of resistance training.
The authors also demonstrated that satellite cells have a more pronounced role in muscle repair during the initial phase of resistance training than in muscle hypertrophy resulting from 10 weeks of resistance training in young men. The role of satellite cells during muscle adaptation to resistance training may differ during muscle hypertrophy. Satellite cells contribute to myonuclear accretion during muscle hypertrophy induced by high-intensity interval training. The myonuclear number was increased by climbing training but not by oestrogen during training.

The myonuclear domain size increases during muscle hypertrophy following resistance training. Ribosomal function is a key regulator of the myonuclear domain size, which was shown to be increased by climbing training in the current study. Oestrogen significantly augmented the climbing training-induced increase in the MHC Ila myonuclear domain size, suggesting that oestrogen exerts fibre type-specific effects. The RNA and protein contents, which are associated with ribosomal function, were higher in the OVX+E-CT group than in the other groups, indicating that oestrogen affects ribosomal function. Augmentation of the myonuclear domain size requires an increase in protein synthesis. Ribosomal function (translational efficiency) and ribosome biogenesis (translational capacity) critically contribute to the regulation of protein synthesis. During skeletal muscle hypertrophy, translational efficiency increases in the initial phase of hypertrophy and translational capacity increases in the late phase of hypertrophy. Several studies reported that ribosome biogenesis is increased in the skeletal muscles following resistance training. We showed that the mRNA and protein levels of rpS6 revealed the main effect of the group, but there was no main effect of training. Hammarström et al. demonstrated that levels of 28S ribosomal RNA, but not those of 18S ribosomal RNA, were significantly increased following 12 weeks of resistance training. The present study also showed that resistance training increased 28S ribosomal RNA levels but not 18S ribosomal RNA levels. The OVX+E-Sed group showed the highest levels of 28S ribosomal RNA among all groups. Stec et al. revealed a ceiling effect on the skeletal muscle ribosome content. These results suggest that oestrogen increases ribosome biogenesis in skeletal muscle; however, ribosome biogenesis may not be responsible for the enhanced training-induced increases in muscle mass and protein in the OVX+E group. This result suggests that oestrogen increases ribosome biogenesis in the skeletal muscle, but the ceiling effect limits any further increases in the OVX+E-CT groups.

In conclusion, oestrogen enhanced the resistance training-induced increase in the myonuclear domain size but did not affect satellite cells and ribosome biogenesis in...
Figure 6 Effects of climbing training and oestrogen on Total RNA and ribosome biogenesis of flexor hallucis longus (FHL) muscle. (A) Total RNA content; (B) mRNA level; (C) total protein level of ribosomal protein S6 (rpS6); (D) 18S and 28S ribosomal RNA (rRNA) content. Values are presented as mean ± SD (n = 6); *P < 0.05. Sham-Sed, sham sedentary; Sham-CT, sham climbing training; OVX-Sed, ovariectomy sedentary; OVX-CT, ovariectomy climbing training; OVX+E-Sed, ovariectomy plus oestrogen treatment sedentary; OVX+E-CT, ovariectomy plus oestrogen treatment climbing training.
hypertrophic muscle. These results provide a foundation for developing strategies aimed at improving muscle mass in postmenopausal women. Although oestrogen may influence the myonuclear domain size to regulate resistance training-induced muscle hypertrophy, the molecular mechanisms remain unclear. The molecular mechanisms might be clarified by RNA-sequencing, RNA turnover, proteomic, and/or muscle hypertrophic-related signalling analysis in the future studies.

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Conflict of interest

Yung-Li Hung, Ayami Sato, Yuka Takino, Akihito Ishigami, and Shuichi Machida declare that they have no conflict of interest exists.
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