Title

Effects of chronic administration of arachidonic acid on lipid profiles and morphology in the skeletal muscles of aged rats

Author(s)

Takayuki Inoue, Michio Hashimoto, Masanori Katakura, Yoko Tanabe, Abdullah Al Mamun, Kentaro Matsuzaki, Hiroki Otani, Osamu Shido

Journal

Prostaglandins, Leukotrienes and Essential Fatty Acids, Volume 91, Issue 4

Published

30 July 2014

URL

https://doi.org/10.1016/j.plefa.2014.07.015

Effects of chronic administration of arachidonic acid on lipid profiles and morphology in the skeletal muscles of aged rats

Takayuki Inoue[a,b], Michio Hashimoto[a,*], Masanori Katakura[a], Yoko Tanabe [a], Abdullah Al Mamun[a], Kentaro Matsuzaki[a], Hiroki Otani[b], Osamu Shido [a]

[a]Department of Environmental Physiology, Faculty of Medicine, Shimane University, 89-1 Enya-cho, Izumo, Shimane, 693-8501, Japan, Tel: +81-853-20-2112, FAX: +81-853-20-2110

[b]Department of Developmental Biology, Faculty of Medicine, Shimane University, 89-1 Enya-cho, Izumo, Shimane, 693-8501, Japan, Tel: +81-853-20-2102, FAX: +81-853-20-2100

*Correspondence to: Michio Hashimoto, Associate Professor, Department of Environmental Physiology, Shimane University Faculty of Medicine, Izumo, Shimane, 693-8501, Japan. Tel: +81-853-20-2112, FAX: +81-853-20-2110, E-mail address: michio1@med.shimane-u.ac.jp.

Source of funding: This study was supported by a Health and Labour Sciences Research Grant of Japan (#H22-Shokuhin-Ippan-002) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (#23500955, MH).

Key words: n-6 fatty acid, oxidative stress, aging, skeletal muscles, myosin heavy chain

ABSTRACT

Arachidonic acid (20:4n-6, ARA) is a major component of the cell membrane, whereas ARA-derived eicosanoids are formed when cells are damaged. Aging is associated with an accretion of oxidative stress in skeletal muscles. In this study, we examined the effects of chronic administration (13 weeks) of ARA (240 mg/kg/day) on fatty acid composition, antioxidative status, and morphology of slow (soleus muscles) and fast (extensor digitorum longus muscles; EDL)-twitch muscles in aged rats (21 months old). The level of reactive oxygen species was higher in the EDL of ARA-administered rats than in that of control rats. ARA administration decreased the muscle cell volumes and increased the number of slow myosin heavy chain (MHC)-positive cells in the EDL. The relative content of MHC2X was increased whereas the relative content of MHC2B was decreased in the EDL of ARA-administered rats. These results suggest that ARA deposition in the fast-twitch muscle of aged rats reduced cell volume with an increase in oxidative stress.

1. Introduction

The age-related loss of skeletal muscle mass and strength, i.e. sarcopenia, is becoming a growing public health problem because it contributes to decreased capacity for independent living [1, 2]. Sarcopenia can be explained by several physiopathological factors, including increased oxidative stress [3]. Aging accretes oxidative stress and increases the incidence of oxidative injury in respiratory and locomotive skeletal muscles [4, 5]. Furthermore, it has been reported that age-related oxidative stress causes a significant decrease in antioxidant enzymes, particularly glutathione peroxidase in rats [6]. Consequently, muscles undergoing elevated levels of oxidative stress develop sarcopenia, which causes considerable age-related decline in muscle mass [7]. On the basis of the myosin heavy chain (MHC) isoform pattern, adult mammalian limb skeletal muscles contain two and, in some species, three types of fast fibers (Type 2A, 2X, and 2B) and one type of slow fiber (Type 1). Fast-twitch muscles such as the extensor digitorum longus muscles (EDL) are composed primarily of a mixture of the fast myosin isozymes, whereas slow-twitch muscles such as the soleus muscle (SO) contain primarily the slow Type 1 fibers [8, 9]. The synthesis rate of the MHC protein is reportedly decreased with age in humans [10, 11]. Moreover, the effects of aging on antioxidant systems in the skeletal muscle may be quite different from those on antioxidant systems in the liver, kidney, brain, and heart [12] because the lipid peroxidation (LPO) level is greater in skeletal muscle homogenate than in other tissue

homogenates [6].

Arachidonic acid (20:4n-6, ARA), a polyunsaturated fatty acid (PUFA) synthesized from linoleic acid (18:2n-6, LA) in many tissues, is a major component of the cell membrane [13, 14]. In various pathophysiological conditions, ARA is released from membrane phopholipids by phospholipases, particularly phospholipase A_2 (PLA_2). Free ARA can be converted to bioactive eicosanoids through the cyclooxygenase (COX), lipoxygenase (LOX), or P-450 epoxygenase pathways [15]. In the skeletal muscle, ARA is necessary for the repair and growth of muscle tissue through its conversion to active components such as prostaglandin (PG) F_2 alpha and COX-2 metabolites [16, 17]. By contrast, PLA_2 activity is increased in patients with Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) [18] and PGE_2 activity is increased in isolated strips of biceps muscle from patients with DMD [19]. Skeletal muscle wasting associated with chronic inflammatory conditions, such as aging-associated sarcopenia in old rats and older adults [20, 21], has been reported to be improved by systemic non-steroidal anti-inflammatory drug (NSAID) treatment. Thus, despite the apparent positive role of the COX/PG pathway in adaptive muscle growth/regeneration *in vivo*, evidence is also indicative of a negative role of this pathway in the maintenance of skeletal muscle mass under conditions of chronic systemic low-grade inflammation.

Despite of these finding, no study has investigated the direct effect of exogenous ARA

availability on morphological changes in the skeletal muscles of aged rats. The present study aimed to examine the impact of supplementation with exogenous ARA on fatty acid composition, lipid peroxidation, and MHC isoform levels in the skeletal muscles of aged rats.

2. Materials and Methods

2. 1. Animals

Five-week-old Wistar (Jcl:Wistar) rats (Generation 0; G0) obtained from Clea Japan (Osaka, Japan) were housed and maintained in an air-conditioned room under a 12-h/12-h light/dark cycle and were given fish oil-deficient food (F1; Funabashi Farm, Funabashi, Japan) and water ad libitum. Breeding commenced when the animals were 3 months of age. The pups (G1 and G2) were maintained under the same conditions as G0. The G2 aged rats were used for the study, which was conducted under the procedures outlined in the Guidelines for Animal Experimentation of Shimane University, compiled from the Guideline for Animal Experimentation of the Japanese Association for Laboratory Animal Science.

2. 2. ARA administration

The G2 male aged rats (100 weeks old) were randomly divided into the ARA group and control group. Rats in the ARA group were intragastrically administered ARA oil [the triglyceride (TG) form of ARA-rich oil: 240 mg/kg body weight (BW)/day) for 13 weeks. The ARA oil was gently emulsified in an ultrasonic homogenizer (Taitec VP-5; Taitec, Tokyo, Japan) just before administration. The control group was administered a similar volume of control oil (beef fat: soybean oil: rape seed oil = 2:1:1) without ARA. The dose of each oil was determined based on previous reports [22, 23]. Administration of the compound was maintained until all experiments were complete. Table 1 shows the composition of the fatty acids in each oil.

2. 3. Blood and muscle sample preparation

Rats were deeply anaesthetized by an intraperitoneal injection of pentobarbital (65 mg/kg BW) and blood was drawn for biochemical assay.

Slow-twitch muscles (SO) and fast-twitch muscles (extensor digitorum longus muscles; EDL) were retrieved from each rat for analysis. Some samples were quickly dissected and immediately frozen in liquid nitrogen for further analysis. Other samples were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C overnight for histological analysis.

2. 4. Lipid analysis

Muscle samples were homogenized in a Polytron homogenizer (PCU-2-110; Kinematica GmbH, Steinhofhalde, Switzerland) in phosphate-buffered saline (1 mL/100-mg tissue) that contained 0.005% (w/v) 2,6-di-t-butyl-4-methylphenol (Wako Chemicals, Osaka, Japan) as an antioxidant. Protein concentrations were estimated using the method of Lowry et al. [24]. Fatty acid levels in the plasma and muscles were prepared and analyzed by a modification of the one-step reaction analysis of Lepage and Roy [25] using gas chromatography (GC) [26]. For each sample, the mixture of plasma or muscle tissue homogenate was augmented with 2 mL of methanol containing 10 μg of tricosanoic acid as an internal standard and 200 μL of acetyl chloride. This mixture was then incubated at 100°C for 60 min, followed by the addition of 200 μL of octane and 5 mL of 10% sodium chloride containing 0.5 N sodium hydroxide. The mixture was shaken for 10 min at room temperature and centrifuged at 2800 × *g* for 15 min. The octane phase, which contained the fatty acid methyl esters, was subjected directly to GC in the Agilent 6850 A gas chromatograph (Agilent Technologies, Santa Clara, CA).

2. 5. Antioxidative–oxidative status

The LPO level was determined using the thiobarbituric acid reactive substances (TBARS) assay [27]. Briefly, 200 μL of 8.1% sodium dodecyl sulfate, 3.0 mL of 0.4% thiobarbituric acid in

20% acetic acid (pH 3.5), and 700 μL of double distilled water were added to 100 μL of homogenate containing 100 μg of protein. The mixture was then incubated for 1 h at 95°C. After cooling in tap water, 1.0 mL of double distilled water and 4.0 mL of n-butanol-pyridine (15:1, v/v) were added and the mixture was shaken vigorously for 20 min. After centrifugation at 1800 × *g* for 10 min, the fluorescence intensity of the upper organic layer was determined with a Hitachi 850 spectrofluorometer (Tokyo, Japan). The excitation and emission wavelengths were 515 and 553 nm, respectively. TBARS levels were expressed as nanomoles of malondialdehyde per milligram of protein. Malondialdehyde levels were calculated relative to a standard preparation of 1,1,3,3-tetraethoxypropane.

The level of reactive oxygen species (ROS) was determined as described previously [28,29]. Briefly, tissue homogenate were centrifuged at 12,500 × *g* for 10 min at 4°C to remove cytosolic proteins. The pellet was suspended at 4°C in 5 mL of 100 mmol/L phosphate buffer (pH 7.4) and mixed with dichlorofluroscein diacetate (Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 5 μmol/L. Fluorescence was monitored with a Hitachi 850 spectrofluorometer at wavelengths of 488 nm for excitation and 525 nm for emission for 60 min at 37°C. ROS levels were quantified from the dichlorofluroscein standard curve and were expressed as moles per min per milligram of protein.

2. 6. RNA isolation and real-time reverse transcription-polymerase chain reaction

Total RNA of the humerus was isolated by Isogen (Wako Pure Chemical Industries), then cDNA was synthesized with the Quantitect reverse transcription kit (Qiagen, Hilden, Germany) and amplified by the Thermal Cycler Dice® Real Time System MRQ (Takara Bio Inc., Shiga, Japan). Real-time reverse transcription (RT) PCR was carried out with the Quantitect SYBR Green PCR kit (Qiagen). Primers for RT-PCR reactions were as follows: rat glutathione peroxidase forward primer (GGAGAATGGCAAGAATGAAGA), reverse primer (CCGCAGGAAGGTAAAGAG); rat superoxide dismutase forward primer (ATCTTCTTGTGCAGTGCCAGC), reverse primer (TAGGGCTCAGGTTTGTCCAG); and rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward primer (GTGACCAGAGCGAAAGCA), reverse primer (CCTTGACTGTGCCGTTGAACT). The PCR conditions were as follows: initial activation at 95°C for 30 seconds, then 40 amplification cycles of denaturation at 95°C for 5 seconds, annealing at 60°C for 31 seconds, and extension at 72°C for 30 seconds. The relative changes in gene expression levels were determined by the $2^{-\Delta\Delta Ct}$ method [30]. All real-time PCR results were normalized to GAPDH.

2. 7. Histological analysis for muscle

Samples were frozen with OCT compound. The tissue blocks were cut in serial sections

(10 μm) that were stained with hematoxylin–eosin (HE) and observed under light microscopy. The serial section in which the maximum area of muscles had appeared was designated as the central section. The previous and subsequent sections were numbered serially in the mediolateral direction. The previous 15 and subsequent 15 sections to the central section were selected for analysis. The region of analysis was 300 μm in size. Other sections were processed for immunohistochemistry with the following characterized antibodies: anti-laminin antibody rabbit IgG (1:2000; Sigma-Aldrich) and anti-slow myosin heavy chain (sMHC) antibody mouse IgG (1:1000; Abcam, Cambridge, UK). The sections were observed by a Pascal confocal laser microscope (Carl Zeiss, Jena, Germany). We chose sections at random from each group, counted the cell number and number of sMHC positive cells, and obtained the number of sMHC-positive cells per cell number.

2. 8. Electrophoretic separation of MHC isoforms

Electrophoresis was conducted under the conditions described by Myzunoya et al. [31]. The muscles were frozen in liquid nitrogen, ground to a powder, and stored at −80°C. Weighed frozen powdered muscles (approximately 30 mg) were homogenized in sodium dodecyl sulfate (SDS) solution containing 10% w/v SDS, 40 mM dithiothreitol (DTT), 5 mM EDTA, and 0.1 M Tris–HCl buffer (pH 8.0) to give a final concentration of muscle tissue of 0.25 mg/μL. These

sample homogenates were heated at 100°C for 3 min. Total protein concentrations were assayed using BCA Protein Assay Reagent (Pierce Biotechnology, Rockford, IL, USA) and standardized with bovine serum albumin. The samples were diluted in 2× sample buffer [100 mM DTT, 4.0% w/v SDS, 0.16 M Tris–HCl (pH 6.8), 43% v/v glycerol, and 0.2% w/v bromophenol blue] and distilled water. The protein concentration was adjusted to 2 mg/mL. The protein samples were frozen at −80°C until further use. The separating gel consisted of 35% v/v glycerol, 8% w/v acrylamide-*N,N′*-methylenebisacrylamide (Bis) (99:1), 0.2 M Tris–HCl (pH 8.8), 0.1 M glycine, 0.4% w/v SDS, 0.1% w/v ammonium persulfate, and 0.05% v/v *N,N,N′,N′*-tetramethylethylenediamine (TEMED). The stacking gel consisted of 30% v/v glycerol, 4% w/v acrylamide-Bis (50:1), 70 mM Tris–HCl (pH 6.7), 4 mM EDTA, 0.4% w/v SDS, 0.1% w/v ammonium persulfate, and 0.05% v/v TEMED. After samples (5 μL) were loaded, electrophoresis was performed at a constant voltage of 140 V for 22 h. After each electrophoresis run, the gels were stained with a silver staining kit (Silver Staining Kit, Protein; GE Healthcare Bio-sciences Corp., Piscataway, NJ, USA).

2. 9. Image analysis

The captured pictures were changed into binary format images using Adobe Photoshop CS2 software. The binary format images were used to measure areas and perimeters with the

Image J software (NIH, Bethesda, MD, USA).

2. 10. Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Data were analyzed using Student's t-test. Correlation was determined by Pearson's correlation analysis. Differences between groups were considered significant at *P*-values less than 0.05. All statistical analyses were performed using PASW Statistics 18.0 (IBM-SPSS, Inc., USA).

3. Results

3. 1. BW and biochemical data

Table 2 shows BW and biochemical data after ARA administration for 13 weeks. The BW of the rats was not affected by ARA administration. Plasma total and low- and high-density lipoprotein cholesterol levels were significantly higher in the ARA group than in the control group ($P < 0.05$). While other biochemical parameters were not affected by ARA administration.

3. 2. Effects of chronic ARA administration on the fatty acid profiles of plasma and skeletal muscles

ARA levels, the n-6/n-3 ratio, and unsaturation index (USI) in the plasma were significantly higher in the ARA group than in the control group ($P < 0.05$) while palmitic acid, LA, and eicosapentaenoic acid (EPA) levels in the plasma were significantly lower in the ARA group than in the control group ($P < 0.05$; Table 3). Oleic acid levels tended to be lower in the ARA group than in the control group ($P = 0.07$; Table 3).

The n-6/n-3 ratio in the SO was significantly higher in the ARA group than in the control group ($P < 0.05$) while docosahexaenoic acid (DHA) levels and the DHA/ARA ratio in the SO

were significantly lower in the ARA group than in the control group ($P < 0.05$). ARA levels in the EDL were significantly higher in the ARA group than in the control group ($P < 0.05$) while LA levels and the DHA/ARA ratio in the EDL were significantly lower in the ARA group than in the control group ($P < 0.05$; Table 4).

Strongly significant positive correlations were observed between plasma LA and EPA levels and LA levels and the EPA/ARA ratio in the EDL. Significant negative correlations were also observed between plasma LA and EPA levels and ARA levels in the EDL (Supplemental Table 1). Similarly, a strongly significant positive correlation was observed between plasma ARA levels and ARA levels in the EDL. A significant negative correlation was also observed between plasma ARA levels and the DHA/ARA and EPA/ARA ratios in the EDL (Supplemental Table 1). The correlation was not observed between plasma ARA levels and the DHA/ARA and EPA/ARA ratios in the SO (data not shown).

3. 3. Effects of chronic ARA administration on oxidative status

TBARS levels in the plasma were significantly higher in the ARA group than in the control group ($P < 0.05$; Table 3), whereas those in the SO were significantly higher in the ARA group than in the control group ($P < 0.05$). Similarly, ROS levels in the EDL were significantly higher in the ARA group than in the control group ($P < 0.05$; Table 4).

Strongly significant negative correlations were observed between plasma TBARS levels and the DHA/ARA and the EPA/ARA ratios in the EDL and between ROS levels and the DHA/ARA ratio in the EDL (Supplemental Table 2). The correlation was not observed between plasma TBARS levels and TBARS levels in the SO and between the DHA/ARA and EPA/ARA ratios in the SO (data not shown).

In slow-twitch muscle, the relative mRNA expressions of superoxide dismutase was lower in the ARA group than in the control group ($P < 0.05$; Fig. 1A). In fast-twitch muscle, the relative mRNA expressions of glutathione peroxidase and superoxide dismutase were significantly lower in the ARA group than in the control group ($P < 0.05$; Fig. 1B).

3. 4. Histological analysis of slow- and fast-twitch muscles

The spaces between the muscle bundles in the EDL were wider in the ARA group than in the control group (Fig. 2B, D and asterisks). Muscle area in the SO was not affected in the ARA group (Fig. 2E); the muscle cell area per muscle area tended to decrease ($0.05 < P < 0.1$) compared with that in the control group (Fig. 2F). On the other hand, muscle area in the EDL was significantly decreased in the ARA group compared with that in the control group ($P < 0.05$; Fig. 2E); the muscle cell area per muscle area was significantly decreased compared with that in the control group ($P < 0.05$; Fig. 2F).

3. 5. Expression and distribution of slow MHC in the fast-twitch muscles

The number of sMHC-positive cells (green, white arrow heads) per muscle cells in the EDL was significantly increased of the ARA group compared with that in the control group ($P < 0.05$; Fig. 3).

3. 6. Changes in MHC isoforms in slow- and fast-twitch muscles

MHC1 expression in the SO was significantly decreased in the ARA group compared with that in the control group ($P < 0.01$) (Fig. 4A and B). MHC2X and MHC2B expression in the EDL were significantly decreased in the ARA group compared with that in the control group ($P < 0.01$; Fig. 4A and C). The relative content of MHC2X in the EDL was increased ($P < 0.01$) while that of MHC2B was decreased ($P < 0.01$) in the ARA group compared with those in the control group (Fig. 4D).

3. 7. Correlations between MHC isoforms and oxidative status in plasma and muscles

Significantly negative correlations were observed between plasma TBARS levels and

MHC1 expression in the EDL, whereas significantly negative correlations were observed between plasma TBARS levels and ROS levels in the EDL and between MHC2X and MHC2B expression in the EDL (Supplemental Table 3).

3. 8. Correlations among cell area, sMHC-positive cells, fatty acid proportion, and oxidative status in plasma and fast-twitch muscles

Significantly negative correlations were observed between cell area in the EDL and plasma (Supplemental Fig. 1B) and EDL (Supplemental Fig. 1C) ARA levels. Inversely significant positive correlations were observed between cell area in the EDL and plasma EPA levels (Supplemental Fig. 1A) and the DHA/ARA ratio in the EDL (Supplemental Fig. 1D). Furthermore, a significantly negative correlation was seen between sMHC-positive cells and the DHA/ARA ratio in the EDL (Supplemental Fig. 1G) while inversely significant positive correlations were observed between sMHC-positive cells and plasma TBARS (Supplemental Fig. 1E) and EDL ARA levels (Supplemental Fig. 1F).

4. Discussion

In the present study, chronic ARA administration was accompanied by a decrease in LA levels and the DHA/ARA ratio and an increase in ARA and ROS levels in the EDL of aged rats. Muscle cell areas were decreased and the number of sMHC-positive cells was increased in the EDL in the ARA group compared with those in the control group. Furthermore, MHC1 expression in the SO was significantly decreased in the ARA group. The relative expression of MHC2X in the EDL was increased while that of MHC2B was decreased in the ARA group compared with those in the control group.

It was reported that ARA-enriched TG oil with or without DHA oil neither affected the health, growth, fertility, or reproductive performance of the parental rats nor affected any pup characteristics (condition, weight gain, viability, number per litter, or sex ratio) [32]. In our experiments, we used the TG form of ARA-rich oil. There was no influence on BW, liver, kidney function index of the blood, and fasting blood glucose levels, suggesting that there was no general systemic toxicity caused by chronic ARA administration. In the present study, plasma total and low- and high-density lipoprotein cholesterol levels were significantly higher in the ARA group than in the control group (Table 2). It was reported that an increase in LA intake lowers plasma cholesterol in humans [33, 34]. Furthermore, Lina et al. reported that the cholesterol concentration was higher in a high-fat control group and an ARA group than in a low-fat control group, while it was lower in an ARA/DHA group than in a high-fat control group [32]. LA levels in the ARA oil

used in this study were less than those in the control oil (Table 1); and LA levels in plasma also decreased in the ARA group (Table 3). Therefore, increases in plasma total and low- and high-density lipoprotein cholesterol levels in ARA-administered aged rats may result from the lower LA levels in the ARA oil. Oleic acid (OLA) levels in the ARA oil used in this study were also less than those in the control oil (Table 1); however OLA levels in the plasma slightly but not significantly decreased in the ARA group because OLA can be sufficiently synthesized in liver or other organs and supply to the plasma.

The fatty acid composition of skeletal muscle varies by muscle fiber type [35-38]; it could be reflected in the content of sarcolemma, sarcoplasmic reticulum, and mitochondria, which have different lipid composition in their membranes [39]. DHA is highly contained in the phospholipid of sarcoplasmic reticulum and mitochondria; therefore, DHA level in the skeletal muscle affects the muscle functions in vitro [40] and in vivo [41]. Stark et al have reported that total n-3 PUFA is comparative between SO and white gastrocnemius; whereas, percentage of DHA in SO significantly lower than that in the white gastrocnemius [42]. In the present study, less DHA percentage in the SO was observed compared with that in the EDL of control group (Table 4); however, it might be reflected in high TG levels are containing in the SO compared with EDL [35]. Mole percentage of DHA in the SO and DHA/ARA ratio in the SO and EDL significantly decreased in the ARA group (Table 4), suggesting that frequency contraction could be decreased by ARA administration. Further study is necessary to confirm muscle volume loss, high oxidative

stress, and switch MHC type were stimulated by either high concentration of ARA or low concentration of DHA in the muscle. In rats, it was reported that ARA composition of phospholipids was lower in the EDL than in the SO [43]. In the present study, less ARA levels in the EDL were also observed than that in the SO of the control group. After chronic ARA administration, ARA levels significantly increased in EDL but not SO. In contrast to ARA, OLA levels in the EDL significantly decreased but not in the SO; LA levels was decreased both in the EDL and the SO. Different transport systems may involve in these difference among fatty acids. Fatty acids transporters or translocators in the muscle regulate fatty acid composition [44, 45]. Guo et al. reported that fatty acid translocase (FAT/CD36) selectively transport ARA or LA but not OLA and palmitic acid [46]. Further studies are needed to determine the difference of these fatty acids transport proteins between the EDL and the SO.

Declines in skeletal muscle mitochondria are thought to play a primary role in age-related progressive loss of muscle mass and muscle strength [47]. The amount of reduced glutathione decreases with aging as a result of functional decline of the mitochondria electron transfer chain [48]. Multiple damages may explain the decrease in respiration rate and activities of different respiratory chain complexes in human skeletal muscles [49, 50]. Low-level ROS plays an important role as the signal transducer of the excitation–contraction coupling; however, high-concentration ROS acts to decrease the function of skeletal muscle [51, 52]. Furthermore, the skeletal muscles are particularly vulnerable to oxidative stress because they are constituted in

postmitotic cells that are liable to accumulate oxidative damage over time [12]. Finally, oxidative stress is increased in the muscles of elderly people in association with cellular lipid, protein, and DNA damage. In the present study, ROS levels significantly increased and the muscle cell volumes decreased in EDL of ARA group. A strongly significant negative correlation was observed between plasma TBARS levels and the DHA/ARA and EPA/ARA ratios in the EDL. Similarly, a strongly significant negative correlation was observed between ROS levels and the DHA/ARA ratio in the EDL. Additionally, the relative mRNA expressions of glutathione peroxidase and superoxide dismutase significantly decreased in EDL of ARA group. These results suggest that increased levels of ARA in the EDL by chronic ARA administration might enhance oxidative stress in EDL.

Skeletal muscle has different fiber-types, which are characterized by the expression of distinct myosin variants. During sarcopenia, there is a decrease in type 2 fibers, with little to no decrease in type 1 fibers [53]. Muscle aging determines the decline in cross-sectional area, fiber denervation, and fiber number loss, primarily type 2 fibers [54, 55]. Type 2 fibers show an age-related decrease in both number and size [56-58]; on the other hand, type 1 fibers increase with age or are little affected [59-562]. In this study, the muscle cell volumes were decreased and the number of sMHC-positive cells was increased in the EDL of ARA-administered aged rats, suggesting that ARA deposition in the EDL of aged rats induced the fiber volume loss.

The synthesis rate of MHC protein decreases with age in humans [8]. mRNA abundance

in MHC2A and 2X fibers was decreased in vastus lateralis biopsy samples from healthy older individuals compared with that in those from healthy younger individuals, whereas the MHC1 mRNA content remained unchanged [63]. In contrast, there is no difference between younger and older individuals in terms of mRNA abundance in MHC isoforms [64]. In this study, MHC1 expression was significantly decreased in the SO of ARA-administered aged rats. Furthermore, the relative content of MHC2X was increased while that of MHC2B was decreased in the EDL of ARA-administered aged rats. These data indicate that ARA deposition in the EDL of aged rats caused a fast-to-slow isoform shift, suggesting that ARA deposition in the skeletal muscle of aged rats accelerates aging process.

In the present study, we investigated whether chronic ARA administration could improve age-related decline of skeletal muscles because ARA or ARA-derived eicosanoids is necessary for the repair and growth of muscle tissue [16, 17]. On the other hands, NSAIDs treatments improved skeletal muscle wasting associated with chronic inflammatory conditions, such as aging-associated sarcopenia in old rats and older adults [20, 21]. Recent our study demonstrated that long-term oral administration of ARA increased ARA-derived eicosanoids level in the plasma of the normal young rats [65]. ARA-derived eicosanoids were not determined in the present study; it could be possible to increase ARA-derived eicosanoids levels in the muscle. Further study is necessary to confirm whether chronic ARA administration induces the production of ARA-derived eicosanoids in the aged rats.

To our knowledge, this is the first demonstration that chronic ARA administration changes in the MHC composition of aging skeletal muscle. The results suggest that chronic ARA administration induces muscle cell volume loss with an increase in oxidative stress in aged rats. In particular, ARA deposition in the EDL of aged rats may induce damage to type 2 muscle cells, such as volume loss.

5. Conclusions

ARA deposition in the type 2 muscle of aged rats reduced cell volume with an increase in oxidative stress. ARA deposition in the EDL also changed a fast-to-slow MHC isoform shift.

Acknowledgments

This study was supported by a Health and Labour Sciences Research Grant of Japan (#H22-Shokuhin-Ippan-002) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (#23500955, MH).

References

1. R. Roubenoff, Origins and clinical relevance of sarcopenia, Can J Appl Physiol 26 (2001) 78-89.
2. I.H. Rosenberg, Sarcopenia: origins and clinical relevance, J Nutr 127 (1997) 990S-991S.

3. C. Howard, L. Ferrucci, K. Sun, L.P. Fried, J. Walston, R. Varadhan, J.M. Guralnik, R.D. Semba, Oxidative protein damage is associated with poor grip strength among older women living in the community, J Appl Physiol (1985) 103 (2007) 17-20.

4. E. Barreiro, C. Coronell, B. Lavina, A. Ramirez-Sarmiento, M. Orozco-Levi, J. Gea, Aging, sex differences, and oxidative stress in human respiratory and limb muscles, Free Radic Biol Med 41 (2006) 797-809.

5. P. Gianni, K.J. Jan, M.J. Douglas, P.M. Stuart, M.A. Tarnopolsky, Oxidative stress and the mitochondrial theory of aging in human skeletal muscle, Exp Gerontol 39 (2004) 1391-1400.

6. L.L. Ji, Antioxidant enzyme response to exercise and aging, Med Sci Sports Exerc 25 (1993) 225-231.

7. E. Carmeli, R. Coleman, A.Z. Reznick, The biochemistry of aging muscle, Exp Gerontol 37 (2002) 477-489.

8. R. Bottinelli, Functional heterogeneity of mammalian single muscle fibres: do myosin isoforms tell the whole story?, Pflugers Arch 443 (2001) 6-17.

9. R.H. Fitts, J.J. Widrick, Muscle mechanics: adaptations with exercise-training, Exerc Sport Sci Rev 24 (1996) 427-473.

10. D.L. Hasten, J. Pak-Loduca, K.A. Obert, K.E. Yarasheski, Resistance exercise acutely increases MHC and mixed muscle protein synthesis rates in 78-84 and 23-32 yr olds, Am J Physiol Endocrinol Metab 278 (2000) E620-E626.

11. P. Balagopal, O.E. Rooyackers, D.B. Adey, P.A. Ades, K.S. Nair, Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans, Am J Physiol 273 (1997) E790-E800.

12. M. Matsuo, F. Gomi, M.M. Dooley, Age-related alterations in antioxidant capacity and lipid peroxidation in brain, liver, and lung homogenates of normal and vitamin E-deficient rats, Mech Ageing Dev 64 (1992) 273-292.

13. P.C. Kam, A.U. See, Cyclo-oxygenase isoenzymes: physiological and pharmacological role, Anaesthesia 55 (2000) 442-449.

14. M. Katori, M. Majima, Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors, Inflamm Res 49 (2000) 367-392.

15. J.E. Kinsella, K.S. Broughton, J.W. Whelan, Dietary unsaturated fatty acids: interactions and possible needs in relation to eicosanoid synthesis, J Nutr Biochem 1 (1990) 123-141.

16. T.A. Trappe, J.D. Fluckey, F. White, C.P. Lambert, W.J. Evans, Skeletal muscle PGF(2)(alpha) and PGE(2) in response to eccentric resistance exercise: influence of ibuprofen acetaminophen, J Clin Endocrinol Metab 86 (2001) 5067-5070.

17. J.F. Markworth, D. Cameron-Smith, Arachidonic acid supplementation enhances in vitro skeletal muscle cell growth via a COX-2-dependent pathway, Am J Physiol Cell Physiol 304 (2013) C56-C67.

18. M. Lindahl, E. Backman, K.G. Henriksson, J.R. Gorospe, E.P. Hoffman, Phospholipase A2

activity in dystrophinopathies, Neuromuscul Disord 5 (1995) 193-199.

19. M.J. Jackson, M.H. Brooke, K. Kaiser, R.H. Edwards, Creatine kinase and prostaglandin E2 release from isolated Duchenne muscle, Neurology 41 (1991) 101-104.

20. I. Rieu, H. Magne, I. Savary-Auzeloux, J. Averous, C. Bos, M.A. Peyron, L. Combaret, D. Dardevet, Reduction of low grade inflammation restores blunting of postprandial muscle anabolism and limits sarcopenia in old rats, J Physiol 587 (2009) 5483-5492.

21. T.A. Trappe, C.C. Carroll, J.M. Dickinson, J.K. LeMoine, J.M. Haus, B.E. Sullivan, J.D. Lee, B. Jemiolo, E.M. Weinheimer, C.J. Hollon, Influence of acetaminophen and ibuprofen on skeletal muscle adaptations to resistance exercise in older adults, Am J Physiol Regul Integr Comp Physiol 300 (2011) R655-R662.

22. H. Oe, T. Hozumi, E. Murata, H. Matsuura, K. Negishi, Y. Matsumura, S. Iwata, K. Ogawa, K. Sugioka, Y. Takemoto, K. Shimada, M. Yoshiyama, Y. Ishikura, Y. Kiso, J. Yoshikawa, Arachidonic acid and docosahexaenoic acid supplementation increases coronary flow velocity reserve in Japanese elderly individuals, Heart 94 (2008) 316-321.

23. Y. Ishikura, G. Ikeda, K. Akimoto, M. Hata, A. Kusumoto, A. Kidokoro, M. Kontani, H. Kawashima, Y. Kiso, Y. Koga, Arachidonic acid supplementation decreases P300 latency and increases P300 amplitude of event-related potentials in healthy elderly men, Neuropsychobiology 60 (2009) 73-79.

24. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, Protein measurement with the

Folin phenol reagent, J Biol Chem 193 (1951) 265-275.

25. G. Lepage, C.C. Roy, Direct transesterification of all classes of lipids in a one-step reaction, J Lipid Res 27 (1986) 114-120.

26. M. Hashimoto, K. Shinozuka, S. Gamoh, Y. Tanabe, M.S. Hossain, Y.M. Kwon, N. Hata, Y. Misawa, M. Kunitomo, S. Masumura, The hypotensive effect of docosahexaenoic acid is associated with the enhanced release of ATP from the caudal artery of aged rats, J Nutr 129 (1999) 70-76.

27. H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal Biochem 95 (1979) 351-358.

28. M.S. Hossain, M. Hashimoto, S. Gamoh, S. Masumura, Antioxidative effects of docosahexaenoic acid in the cerebrum versus cerebellum and brainstem of aged hypercholesterolemic rats, J Neurochem 72 (1999) 1133-1138.

29. M. Hashimoto, M.S. Hossain, T. Shimada, H. Yamasaki, Y. Fujii, O. Shido, Effects of docosahexaenoic acid on annular lipid fluidity of the rat bile canalicular plasma membrane, J Lipid Res 42 (2001) 1160-1168.

30. K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402-408.

31. W. Mizunoya, J. Wakamatsu, R. Tatsumi, Y. Ikeuchi, Protocol for high-resolution separation of rodent myosin heavy chain isoforms in a mini-gel electrophoresis system, Anal Biochem

377 (2008) 111-113.

32. B.A. Lina, A.P. Wolterbeek, Y. Suwa, S. Fujikawa, Y. Ishikura, S. Tsuda, M. Dohnalek, Subchronic (13-week) oral toxicity study, preceded by an in utero exposure phase, with arachidonate-enriched triglyceride oil (SUNTGA40S) in rats, Food Chem Toxicol 44 (2006) 326-335.

33. D.F. Horrobin, Y.S. Huang, The role of linoleic acid and its metabolites in the lowering of plasma cholesterol and the prevention of cardiovascular disease, Int J Cardiol 17 (1987) 241-255.

34. G. Rassias, M. Kestin, P.J. Nestel, Linoleic acid lowers LDL cholesterol without a proportionate displacement of saturated fatty acid, Eur J Clin Nutr 45 (1991) 315-320.

35. J. Gorski, A. Nawrocki, M. Murthy, Characterization of free and glyceride-esterified long chain fatty acids in different skeletal muscle types of the rat, Mol Cell Biochem 178 (1998) 113-118.

36. W.G. Blackard, J. Li, J.N. Clore, W.B. Rizzo, Phospholipid fatty acid composition in type I and type II rat muscle, Lipids 32 (1997) 193-198.

37. A.D. Kriketos, D.A. Pan, J.R. Sutton, J.F. Hoh, L.A. Baur, G.J. Cooney, A.B. Jenkins, L.H. Storlien, Relationships between muscle membrane lipids, fiber type, and enzyme activities in sedentary and exercised rats, Am J Physiol 269 (1995) R1154-R1162.

38. M.G. Nikolaidis, A. Petridou, V. Mougios, Comparison of the phospholipid and

triacylglycerol fatty acid profile of rat serum, skeletal muscle and heart, Physiol Res 55 (2006) 259-265.

39. W. Fiehn, J.B. Peter, J.F. Mead, M. Gan-Elepano, Lipids and fatty acids of sarcolemma, sarcoplasmic reticulum, and mitochondria from rat skeletal muscle, 246 (1971) 5617-5620.

40. K.J. Ayre, A.J. Hulbert, Effects of changes in dietary fatty acids on isolated skeletal muscle functions in rats, 80 (1996) 464-471.

41. Infante, R.C. Kirwan, J.T. Brenna, High levels of docosahexaenoic acid (22:6n-3)-containing phospholipids in high-frequency contraction muscles of hummingbirds and rattlesnakes, 130 (2001) 291-298.

42. K.D. Stark, S.Y. Lim, N. Jr Salem, Docosahexaenoic acid and n-6 docosapentaenoic acid supplementation alter rat skeletal muscle fatty acid composition, 6 (2007) 13S.

43. L. St Claire, Evaluating a workshop designed to help medical students improve their skills in communicating about cancer: methodological considerations--and worries, Med Teach 14 (1992) 65-76.

44. E. Harasim, A. Kalinowska, A. Chabowski, T. Stepek, [The role of fatty-acid transport proteins (FAT/CD36, FABPpm, FATP) in lipid metabolism in skeletal muscles], Postepy Hig Med Dosw (Online) 62 (2008) 433-441.

45. A. Chabowski, J. Gorski, J.J. Luiken, J.F. Glatz, A. Bonen, Evidence for concerted action of FAT/CD36 and FABPpm to increase fatty acid transport across the plasma membrane,

Prostaglandins Leukot Essent Fatty Acids 77 (2007) 345-353.

46. J. Guo, G. Shu, L. Zhou, X. Zhu, W. Liao, S. Wang, J. Yang, G. Zhou, Q. Xi, P. Gao, Y. Zhang, S. Zhang, L. Yuan, Q. Jiang, Selective transport of long-chain fatty acids by FAT/CD36 in skeletal muscle of broilers, Animal 7 (2013) 422-429.

47. C.M. Peterson, D.L. Johannsen, E. Ravussin, Skeletal muscle mitochondria and aging: a review, J Aging Res 2012 (2012) 194821.

48. R.H. Hsieh, J.H. Hou, H.S. Hsu, Y.H. Wei, Age-dependent respiratory function decline and DNA deletions in human muscle mitochondria, Biochem Mol Biol Int 32 (1994) 1009-1022.

49. G. Benzi, O. Pastoris, F. Marzatico, R.F. Villa, F. Dagani, D. Curti, The mitochondrial electron transfer alteration as a factor involved in the brain aging, Neurobiol Aging 13 (1992) 361-368.

50. I. Trounce, E. Byrne, S. Marzuki, Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing, Lancet 1 (1989) 637-639.

51. F.H. Andrade, M.B. Reid, D.G. Allen, H. Westerblad, Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse, J Physiol 509 (1998) 565-575.

52. F.H. Andrade, M.B. Reid, H. Westerblad, Contractile response of skeletal muscle to low peroxide concentrations: myofibrillar calcium sensitivity as a likely target for redox-modulation, FASEB J 15 (2001) 309-311.

53. T.J. Doherty, Invited review: Aging and sarcopenia, J Appl Physiol 95 (2003) 1717-1727.

54. F. Jakobsson, K. Borg, L. Edstrom, Fibre-type composition, structure and cytoskeletal protein location of fibres in anterior tibial muscle. Comparison between young adults and physically active aged humans, Acta Neuropathol 80 (1990) 459-468.

55. J. Lexell, Human aging, muscle mass, and fiber type composition, J Gerontol A Biol Sci Med Sci 50 Spec No (1995) 11-16.

56. A.R. Coggan, R.J. Spina, M.A. Rogers, D.S. King, M. Brown, P.M. Nemeth, J.O. Holloszy, Histochemical and enzymatic characteristics of skeletal muscle in master athletes, J Appl Physiol (1985) 68 (1990) 1896-1901.

57. L. Edstrom, L. Larsson, Effects of age on contractile and enzyme-histochemical properties of fast- and slow-twitch single motor units in the rat, J Physiol 392 (1987) 129-145.

58. A. Ishihara, H. Naitoh, S. Katsuta, Effects of ageing on the total number of muscle fibers and motoneurons of the tibialis anterior and soleus muscles in the rat, Brain Res 435 (1987) 355-358.

59. T. Ansved, L. Edstrom, Effects of age on fibre structure, ultrastructure and expression of desmin and spectrin in fast- and slow-twitch rat muscles, J Anat 174 (1991) 61-79.

60. D. Danieli-Betto, R. Betto, A. Megighian, M. Midrio, G. Salviati, L. Larsson, Effects of age on sarcoplasmic reticulum properties and histochemical composition of fast- and slow-twitch rat muscles, Acta Physiol Scand 154 (1995) 59-64.

61. T.J. Eddinger, R.G. Cassens, R.L. Moss, Mechanical and histochemical characterization of skeletal muscles from senescent rats, Am J Physiol 251 (1986) C421-C430.

62. T.J. Eddinger, R.L. Moss, R.G. Cassens, Fiber number and type composition in extensor digitorum longus, soleus, and diaphragm muscles with aging in Fisher 344 rats, J Histochem Cytochem 33 (1985) 1033-1041.

63. S. Welle, K. Bhatt, C. Thornton, Polyadenylated RNA, actin mRNA, and myosin heavy chain mRNA in young and old human skeletal muscle, Am J Physiol 270 (1996) E224-E229.

64. P. Balagopal, J.C. Schimke, P. Ades, D. Adey, K.S. Nair, Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise, Am J Physiol Endocrinol Metab 280 (2001) E203-E208.

65. Juman, M. Hashimoto, M. Katakura, T. Inoue, Y. Tanabe, M. Arita, T. Miki, O. Shido, Effects of long-term oral administration of arachidonic acid and docosahexaenoic acid on the immune functions of young rats, 5 (2013) 1949-1961.

Table 1 - Composition of the fatty acid in administrated oils

(mol%)	Control oil	ARA oil
Palmitic acid C16:0	13.8±0.01	6.95±0.00
Stearic acid C18:0	13.8±0.01	5.91±0.00
Oleic acid C18:1n-9	42.5±0.03	5.31±0.00
Linoleic acid C18:2n-6	20.0±0.02	9.38±0.01
Arachidonic acid C20:4n-6	ND	45.1±0.04
Eicosapentaenoic acid C20:5n-3	0.13±0.01	0.52±0.00
Docosapentaenoic acid C22:5n-3	ND	ND
Docosahexaenoic acid C22:6n-3	ND	ND

ND: Not detected

Table 2 - Body weight and biochemical parameters of plasma

	Control	ARA
Number of rats	6	6
Body weight (g)	404±13	419±10
GOT (IU/L)	84.5±8.7	84.3±9.6
GPT (IU/L)	44.5±3.3	49.3±4.0
γ-GTP (IU/L))	1.5±0.2	1.8±0.3
Albumin (mg/dL)	2.70±0.04	2.63±0.07
Total cholesterol (mg/dL)	98.7±5.2	116.0±4.6*
Triglyceride (mg/dL)	56.7±14.8	36.5±5.2
Blood urea nitrogen (mg/dL)	17.2±1.2	19.7±0.7
Creatinine (mg/dL)	0.27±0.02	0.29±0.01
Blood glucose (mg/dL)	122.0±5.6	126.8±4.3
HDL cholesterol (mg/dL)	57.3±4.1	70.2±1.8*
LDL cholesterol (mg/dL)	28.3±0.8	35.0±2.8*

Data are the mean ± SEM. *$P < 0.05$

GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GTP γ-glutamyl transpeptidase, HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol.

Table 3 - Fatty acid profiles and oxidative status in plasma

	Control	ARA
Number of rats	6	6
Palmitic acid C16:0 (mol%)	24.3±0.5	22.7±0.5*
Stearic acid C18:0 (mol%)	14.1±0.3	14.6±0.4
Oleic acid C18:1n-9 (mol%)	10.2±0.7	8.7±0.6
Linoleic acid C18:2 n-6 (mol%)	18.1±1.2	14.2±0.9*
Arachidonic acid C20:4n-6 (ARA) (mol%)	28.7±1.9	36.3±1.7*
Eicosapentaenoic acid C20:5n-3 (EPA) (mol%)	0.69±0.12	0.36±0.09*
Docosapentaenoic acid C22:5n-3 (mol%)	0.51±0.12	0.44±0.09
Docosahexaenoic acid C22:6n-3 (DHA) (mol%)	1.79±0.27	1.33±0.25
n-6/n-3	15.9±2.8	24.1±3.6
DHA/ARA	0.06±0.01	0.04±0.01
EPA/ARA	0.025±0.005	0.011±0.004*
Unsaturation index (USI)	179.5±4.3	195.3±3.8*

TBARS (nmol/mL)	9.4±0.6	13.7±0.7*

Data are the mean ± SEM. *$P < 0.05$

TBARS, thiobarbituric acid reactive substance.

Table 4 - Fatty acid profiles and oxidative status in the muscle fibers

	SO		EDL	
	Control	ARA	Control	ARA
Number of rats	6	6	6	6
Palmitic acid C16:0 (mol%)	19.40±0.96	21.31±0.68	26.30±0.67	27.50±0.55
Stearic acid C18:0 (mol%)	18.38±1.28	16.15±1.67	13.68±0.59	14.12±0.38
Oleic acid C18:1n-9 (mol%)	12.54±2.32	15.87±2.54	12.31±0.91	10.23±0.57
Linoleic acid C18:2 n-6 (mol%)	27.36±0.53	24.54±1.45	25.61±0.50	20.30±0.79**
Arachidonic acid C20:4n-6 (ARA) (mol%)	15.16±1.44	17.07±2.26	11.63±0.64	18.40±0.71**
Eicosapentaenoic acid C20:5n-3 (EPA) (mol%)	0.102±0.004	0.077±0.015	0.100±0.004	0.089±0.016
Docosapentaenoic acid C22:5n-3 (mol%)	1.19±0.13	0.93±0.15	1.43±0.06	1.48±0.03
Docosahexaenoic acid C22:6n-3 (DHA) (mol%)	5.20±0.54	3.39±0.50*	8.31±0.81	7.34±0.38
n-6/n-3	6.61±0.57	9.46±0.91*	3.80±0.28	4.31±0.19
DHA/ARA	0.35±0.02	0.20±0.01**	0.71±0.04	0.40±0.01**
EPA/ARA	0.007±0.001	0.006±0.002	0.009±0.001	0.005±0.001**

Unsaturation index (USI)	166.3±6.6	159.5±7.1	168.4±5.4	145.7±1.0
TBARS (nmol/mg of protein)	1.37±0.21	2.83±0.57*	3.44±0.84	3.99±0.19
ROS (nmol/min/mg of protein)	3.88±0.49	2.56±0.72	2.84±0.71	5.86±0.68*

Data are the mean ± SEM. $^{**}P < 0.01$, $^{*}P < 0.05$

SO, slow-twitch muscle; EDL, fast-twitch muscle; TBARS, thiobarbituric acid reactive substance; ROS, reactive oxygen species.

Captions to illustrations

Fig. 1. The relative mRNA expressions of antioxidant enzyme in the skeletal muscles

SO, slow-twitch muscle; EDL, fast-twitch muscle; GPx, glutathione peroxidase; SOD, superoxide dismutase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. (A) The relative mRNA expressions of antioxidant enzyme in the slow-twitch muscle. (B) The relative mRNA expressions of antioxidant enzyme in the fast-twitch muscle. Data are expressed as mean ± SEM (n = 6). $^{**}P < 0.01$, $^{*}P < 0.05$.

Fig. 2. Histological changes in muscles

SO, slow-twitch muscle; EDL, fast-twitch muscle. (A) The cross-section of SO in the control group. (B) The cross-section of EDL in the control group. (C) The cross-section of SO in the ARA

group. (D) The cross-section of the EDL in the ARA group. Spaces between muscle bundles in the EDL were wider (B and D, asterisks) in the ARA group than in the control group. (E) The muscle area of the SO and EDL in each group. (F) The muscle cell area per muscle area of SO and EDL in each group. Scale bars: 200 μm. Data are expressed as mean ± SEM (n = 6). *$P < 0.05$.

Fig. 3. Analysis of expression and distribution of slow myosin heavy chain in the fast-twitch muscles using immnohistochemistry

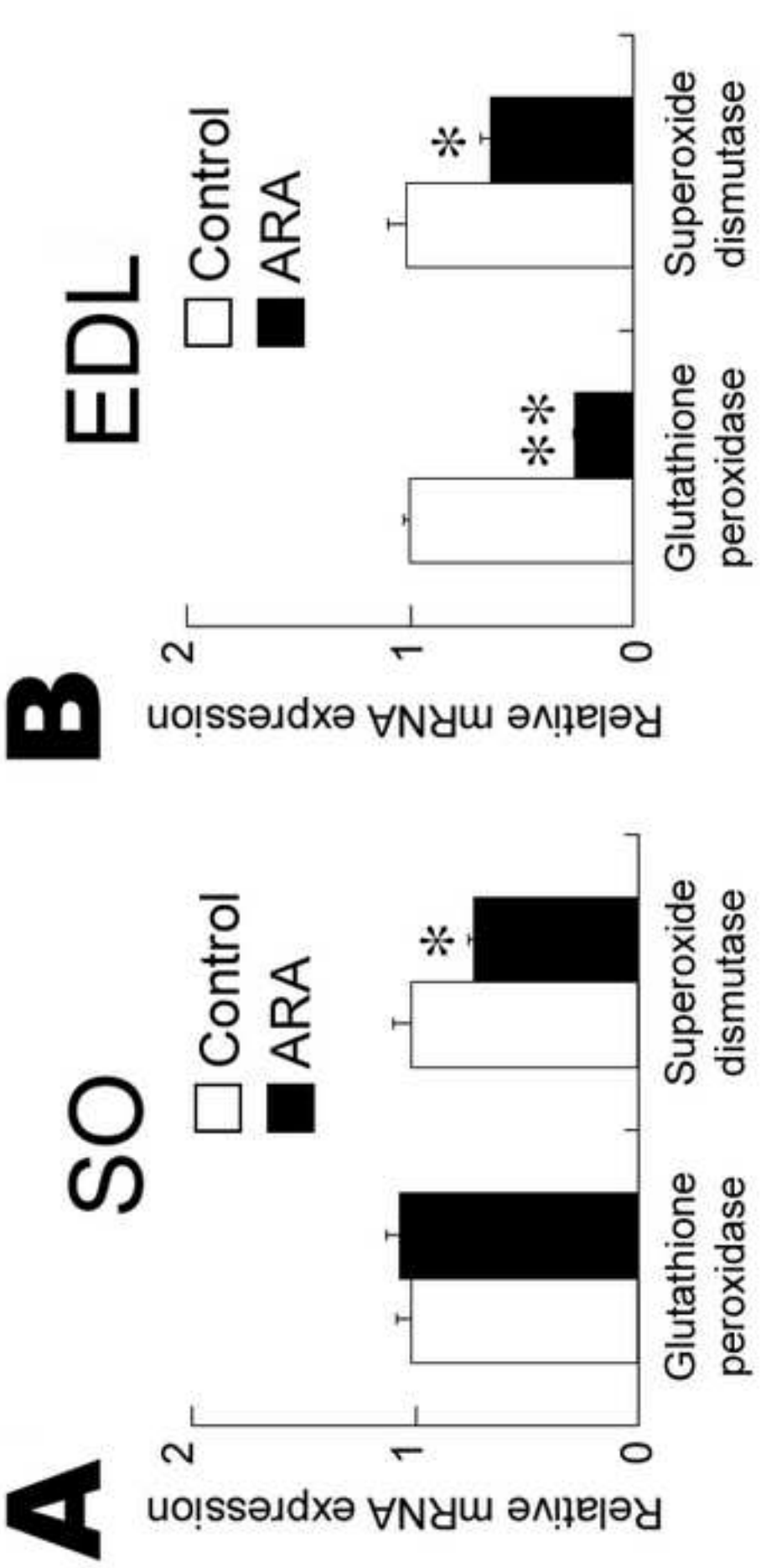
sMHC, slow myosin heavy chain. (A) Immunohistochemistry image of the fast-twitch muscles (EDL) in the control group. (B) Immunohistochemistry image of the EDL in the ARA group. The green color represents sMHC-positive cells (white arrow heads). The red color represents laminin (as a marker of the basement membrane). (C) sMHC-positive cells per cell number. Scale bars: 100 μm. Data are expressed as mean ± SEM (n = 6). *$P < 0.05$.

Fig. 4. Representative electrophoretic gel separation of myosin heavy chain isoforms in slow- and fast-twitch muscles

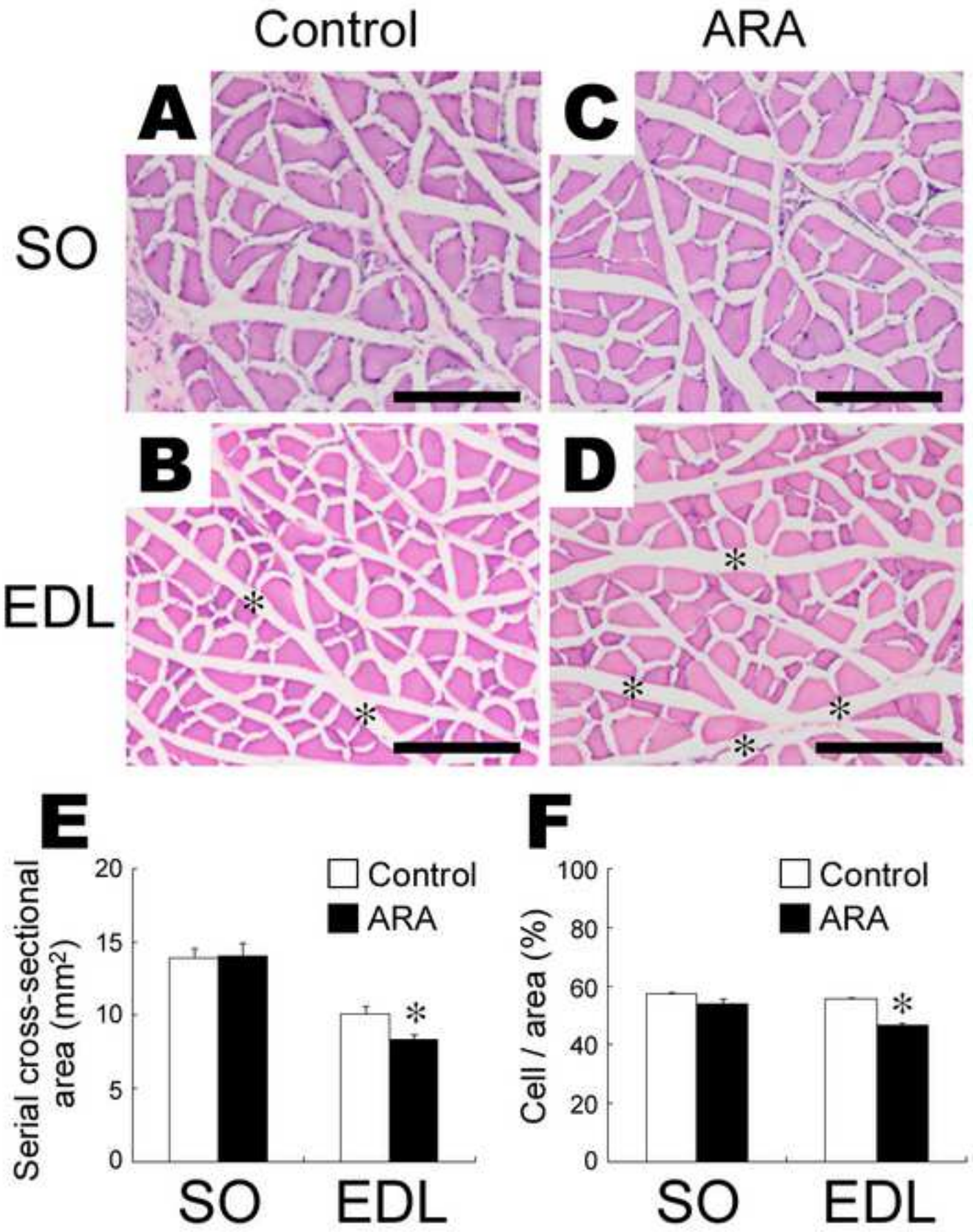
SO, slow-twitch muscle; EDL, fast-twitch muscle; MHC, myosin heavy chain. (A) Scanned image of electrophoretic gel separation of MHC isoforms. (B, C) Plot of the peak areas for the gel as shown in SO (B) and EDL (C). (D) MHC isoform ratios in the EDL. Data are expressed as mean ± SEM (n = 6). **$P < 0.01$.

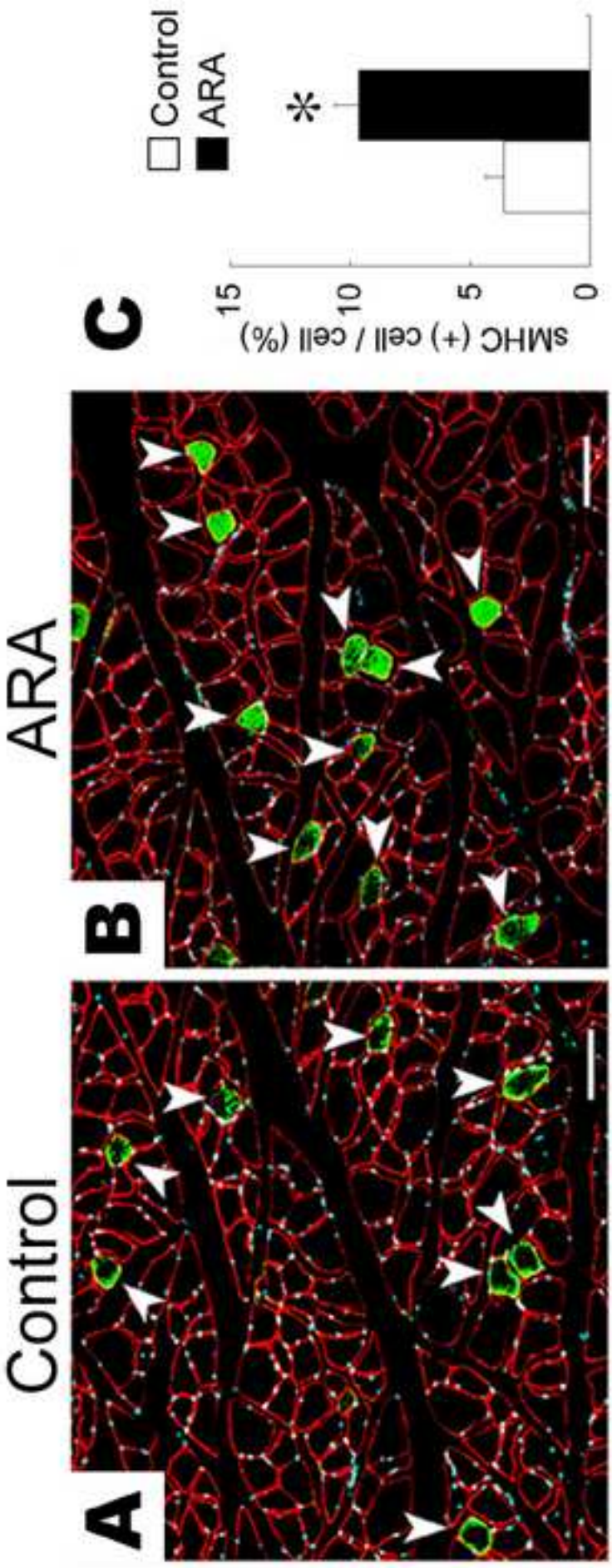
HIGHLIGHTS

1. Arachidonic acid (ARA) administration to aged rats increases ARA levels in the fast-twitch muscles.
2. ARA administration increases reactive oxygen species level in the fast-twitch muscle.
3. ARA administration decreases the muscle cell volumes in the fast-twitch muscle.
4. Slow myosin heavy chain-positive cell number is increased in the fast-twitch muscles.
5. ARA decreases muscle cells volume and type 2 muscle cells.



Figure





Figure

Figure