Title

Differential effects of docoosahexaenoic and arachidonic acid on fatty acid composition and myosin heavy chain-related genes of slow- and fast-twitch skeletal muscle tissues

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Differential effects of docoosahexaenoic and arachidonic acid on fatty acid composition and myosin heavy chain-related genes of slow- and fast-twitch skeletal muscle tissues

Abstract

Myosin heavy chain (MHC) mediates the metabolic and contractile responses of skeletal muscles. MHC displays different isoforms, each of which has different characteristics. To better understand the effect of polyunsaturated fatty acids in skeletal muscles, rats were fed with control-, docosahexaenoic acid (DHA)- and arachidonic acid (ARA)-oil, and the effects on plasma and muscular fatty acid profile, oxidative stress, mRNA levels of myosin heavy chain isoforms MHC1 of slow-twitch muscle (SO) and MHC2A, MHC2X and MHCB isoforms of extensor digitorum longus (EDL) of fast-twitch muscle were evaluated. Concomitantly, mRNA levels of anti-oxidative enzymes, such as, catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD were determined. The expressions of MHC1, MHC2A, MHC2X and MHC2B were lower in the SO of the DHA-fed rats. In the EDL muscles of DHA-fed rats, the expressions of MHC1 and MHC2A increased, however, the expressions of MHC2X increased and that of the MHC2 were not altered. Oxidative stress, as indicated by the levels of LPO, was significantly higher in the plasma of the ARA-fed rats, when compared with that of the DHA-fed rats. The LPO levels were higher both in the SO and EDL muscles of ARA-fed rats. Compared with ARA oil intake, DHA oil showed higher mRNA levels of GPx and SOD. Catalase expression was higher only in the EDL but not in the SO type muscles. Our studies finally indicate that DHA and ARA differentially affect the regulation of contractile and metabolic properties of slow- and fast-twitch skeletal muscles.

Key word: Skeletal muscle, Myosin Heavy Chain isoforms, Anti-inflammatory effect, Arachidonic acid, Docosahexaenoic acid

Introduction

Skeletal muscle is the largest organ in the human body and comprises approximately 40% of total body weight. 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 [1,2]. Fiber type composition varies greatly between individuals, resulting in differences in exercise performance, fatigue resistance, and metabolic capacity in humans [3]. The muscle fiber type in adult muscles can switch in response to activation/contraction patterns, e.g., external electrical stimulation [4], denervation [5], mechanical unloading [6] and food components [7].

Polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA, C20:5, n-3) and arachidonic acid (ARA, C20:4, n-6) are involved in the inflammatory process. Inflammatory cytokines and tumor cells induce PUFA-metabolizing enzymes such as secreted phospholipase A2 (sPLA2) and cyclooxygenase (COX)-2 [8]. DHA and EPA, which are omega-3 fatty acids, have a variety of anti-inflammatory and immune-modulating effects. In addition, these fatty acids are oxidized by COX, lipoxygenase (LOX), or cytochrome P450 monooxygenases to produce DHA-derived docosanoids and EPA-derived eicosanoids, which have anti-inflammatory effects [9]. Various biological effects of omega-3 fatty acids have been demonstrated in several feeding studies with humans and animals using fish or fish oil supplements. The anti-obesity effect of n-3 PUFA reflects the metabolic changes in several tissues, such as stimulation of lipid oxidation and inhibition of lipogenesis in the liver [10, 11], stimulation of fatty acid oxidation in the muscle [12].

ARA, a PUFA synthesized from its precursor linoleic acid (18:2n-6, LA) in many tissues, is a major component of the cell membrane [13, 14]]. In various (patho)physiological conditions, ARA is released from membrane phospholipids by phospholipases, particularly PLA_2. Free ARA can be converted to bioactive eicosanoids through the COX, 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

F_{2alpha} (PGF2α) and COX-2 metabolites [16, 17]. Skeletal muscle wasting associated with chronic inflammatory conditions, such as aging-associated sarcopenia in old rats and older adults [18, 19] has been reported to be improved by systemic non-steroidal anti-inflammatory drug (NSAID) treatment. In recent study, it was reported that dietary fat affects the expression of genes related to the contractile and metabolic properties in the fast-type dominant skeletal muscle, where the activation of oxidative metabolism is more pronounced after fish oil intake than that after soybean oil intake [20]. Additionally, we reported that ARA deposition in the fast-twitch muscle of aged rats reduced cell volume with an increase in oxidative stress [21]. Since the composition of the muscle fiber types closely reflects its metabolic property, it is important to determine whether the muscle fiber types are affected by different types of dietary fat. If this were indeed the case, a diet composition that enhances aerobic metabolism would result in higher numbers of slow or intermediate-type fibers. In this study, we examined whether different PUFA; DHA and ARA could affect the composition and metabolism-related genes in skeletal muscle fibers in both slow- and fast-twitch muscle tissues.

Materials and Methods

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 young rats (5 weeks old) 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.

Oil administration

Table 1 shows the fatty acid composition of each dietary oil. The oil administrations were determined as described previously [22]. The G2 young rats (5 weeks old) were randomly divided into three groups: control group, DHA group (the triglyceride form of DHA rich oil: 240 mg/kg BW/day), and ARA group (the triglyceride form of ARA rich oil: 240 mg/kg BW/day). Control rats were administered a basic mixture of oil (beef fat: soybean oil: rape seed oil = 2:1:1). Each oil mixture was orally administered to the rats (n = 8) for 13 weeks. The dose of oil was determined based on the previous reports [23, 24]. Administration of the oil was maintained until all experiments were completed.

Blood and muscle sample preparation

Rats were deeply anaesthetized by an intraperitoneal injection of pentobarbital (65 mg/kg BW) and blood was drawn for further analysis. Slow-twitch muscles (SO) and fast-twitch muscles (extensor digitorum longus muscles; EDL) were collected from each rat for analysis. Muscle samples were cut in the center of muscles. 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, embedded in paraffin, and cut into serial sections (5 μm) for histological analysis. The other samples were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C overnight, transferred to 20% sucrose in phosphate buffer for cryoprotection. These samples were frozen with OCT compound, and cut into frozen serial sections (10 μm) for Sudan Black-B staining.

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),

containing 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. (1951) [25]. Fatty acid profiles of the plasma and muscles were prepared and analyzed by a modification of the one-step reaction analysis of Lepage and Roy (1986) [26] using gas chromatography (GC) [27]. For each sample, the mixture of plasma and/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).

Antioxidative–oxidative status

The LPO level was determined using the thiobarbituric acid reactive substances (TBARS) assay [28]. 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.

RNA isolation and real-time reverse transcription-polymerase chain reaction

Total RNA of the muscles 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). The primer sequences are listed in Table 2. 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 [31]. All real-time PCR results were normalized to GAPDH.

Histological analysis and analysis of cell types in skeletal muscles

Histological analysis was determined as described previously by Inoue et al., 2014 [21]. The serial paraffin sections were stained with hematoxylin–eosin (HE) and observed under light microscopy. The 10 sections were selected for analysis. The serial frozen sections were stained with Sudan Black-B for analysis of cell types in skeletal muscles and observed under light microscopy. The 10 sections were selected for analysis. Sudan Black-B staining was performed basically as previously described [32, 33].

Electrophoretic separation of MHC isoforms

Electrophoresis was determined as described previously by Inoue et al. (2014) [21]. 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).

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).

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). The baseline characteristics of the control, DHA, and ARA groups were compared using one-way analysis of variance (ANOVA) for continuous variables and Scheffe's *post hoc* test for categorical variables. A *p* value less than 0.05 was considered significant. All statistical analyses were

performed with PASW Statistics 18.0 (IBM-SPSS, Armonk, NY, USA).

Results

Body weight data

The body weight of the rats was not affected in either of the oil administered-groups [BW: Control, 417.7±9.0 g; DHA-oil group, 444.4±5.5 g; ARA-oil group, 414.6±10.0 g].

Effects of chronic oil administration on the fatty acid profiles of plasma and skeletal muscles

Table 3 shows fatty acid profiles in plasma. Unsaturation index (USI) in the plasma was significantly lower in the control group than those in the DHA and ARA group ($P < 0.05$). LA level in the plasma were significantly lower in the ARA group, when compared with those in the control and DHA groups ($P < 0.05$). The n-6/n-3 ratio in the plasma was significantly higher ($P < 0.05$) in the ARA group than those in the control and DHA groups. DHA levels and the DHA/ARA ratios in the plasma of control and ARA groups were significantly lower than those in the DHA group ($P < 0.05$). Oleic acid levels were lower in the DHA and ARA group than in the control group, while SCD index were lower in the DHA and ARA group than in the control group ($P = 0.05$).

Table 4 shows fatty acid profiles in slow-twitch muscles. USI in the SO was significantly lower in the ARA group than in the control and DHA group ($P < 0.05$). The n-6/n-3 ratio in the SO was the highest in the ARA group ($P < 0.05$), while DHA levels and the DHA/ARA ratio in the SO were the lowest in the ARA group ($P < 0.05$). EPA levels in the SO were significantly higher in the DHA group than in the control and ARA group ($P < 0.05$), while the EPA/ARA ratio in the SO are significantly lower in the control and ARA group than in the DHA group ($P < 0.05$).

Table 5 shows fatty acid profiles in fast-twitch muscles. USI in the EDL was significantly

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

Strongly significant positive correlations were observed between plasma LA, EPA and ARA levels and DHA levels and the DHA/ARA ratio in the SO. Significant negative correlations were also observed between plasma LA, EPA and ARA levels and the n-6/n-3 ratio in the SO (Table 6). Similarly, a strongly significant positive correlation was observed between plasma LA, EPA and ARA levels and DHA levels and the DHA/ARA ratio 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 (Table 6).

Effects of chronic each oil administration on oxidative status

TBARS levels, which reflect the levels of lipid peroxide (LPO), in the plasma were significantly higher in the ARA group than in the DHA group ($P < 0.05$; Table 3). Similarly, TBARS levels in the slow-twitch were significantly higher in the ARA group than in the DHA group ($P < 0.05$; Table 4). However, the levels of TBARS were not significantly different in the fast-twitch muscles of either group (Table 5). Strongly significant negative correlations were observed between plasma TBARS levels and DHA levels, the DHA/ARA and the EPA/ARA ratios in the SO and between TBARS levels in the SO and the ARA levels and n6/n3 ratio in the SO (Table 6).

Similarly, strongly significant negative correlations were observed between plasma TBARS levels and DHA levels, the DHA/ARA and the EPA/ARA ratios in the EDL and between LOS levels in the EDL and the DHA levels, the DHA/ARA and the EPA/ARA ratios in the EDL (Table 7).

In slow-twitch muscle (SO), the relative mRNA expression of superoxide dismutase was lower in the ARA group than in the control and DHA group ($P < 0.05$; Fig. 1). The mRNA expression of catalase was the lowest in the SO of the DHA group, whereas those in the SO of the ARA group it was the highest ($P < 0.05$; Fig. 1). Similarly, the relative mRNA expression of glutathione peroxidase was higher in the DHA group than in the control and ARA group ($P < 0.05$; Fig. 1). In fast-twitch muscle, the relative mRNA expressions of glutathione peroxidase, catalase and superoxide dismutase were significantly lower in the ARA group than those in the control and DHA group ($P < 0.05$; Fig. 1).

Histological analysis of slow- and fast-twitch muscles

Muscle areas in the SO and EDL were not affected in each group (Fig. 2G). The muscle cell areas per muscle area in the SO were not affected in each group, whereas those in the EDL were decreased in the ARA group compared with that in the control and DHA group (Fig. 2H).

Histological changes of muscle cell types in slow- and fast-twitch muscles

Ratio of red muscle cell number per muscle cell number in the SO was higher in the ARA group, whereas that was significantly lower in the DHA group than in the control and ARA group (Fig. 3G). Red and inter mediate muscle cell mean areas per muscle cell mean areas was higher in the DHA group than in the control and ARA group, whereas that was significantly lower in the ARA group than in the control and DHA group (Fig. 3H).

Changes of MHC isoforms in slow- and fast-twitch muscles

Ratios of MHC2X and MHC2B in the SO were significantly decreased in the DHA and ARA group compared with those in the control group ($P < 0.05$; Fig. 4A), additionally these were significantly decreased in the DHA compared with those in the DHA group ($P < 0.05$; Fig. 4A).

The relative mRNA expressions of MHC1, MHC2A and MHC2B in the SO were significantly lower in the DHA group than in the control and ARA group ($P < 0.05$; Fig. 4B). Ratios of MHC1 and MHC2A in the EDL were significantly increased in the DHA group compared with those in the control and ARA group ($P < 0.05$; Fig. 4A). The relative mRNA expressions of MHC1 and MHC2A in the EDL were highest in the DHA group ($P < 0.05$; Fig. 4B) while these were lowest in the ARA group.

Discussion

In the present study, chronic oral administrations of DHA- and ARA-oil were accompanied by significant increases in the levels of DHA and ARA, respectively, in the plasma, slow-twitch and fast-twitch muscle fibers, thus suggesting that a clear cut bioavailability of these polyunsaturated fatty acids occurred via intestinal absorption. A significant decrease in the n-6/n-3 ratio and an increase in the DHA/ARA ratios, which were found in the SO muscles of the DHA-fed rats, appear to ameliorate the anti-oxidative potential of the slow-twitch muscles of these rats. The speculation is based on the fact that n-3 PUFAs, particularly DHA significantly increased (1.4-fold as compared to the controls, >4-fold as compared to the ARA-fed rats) in the SO muscle fibers of the DHA-fed rats. In contrast, the decreases in the levels of n-3 fatty acids (EPA+DHA+DPA) led to a vigorous upsurge of the n6/n3 molar ratio in the ARA-fed rats. We have previously reported that the n6/n3 molar ratio, particularly DHA/ARA molar ratio could act as one of the indices of oxidative stress in the brain tissues of both the young (Gamoh et al., 2001) [34] and old rats (Hossain et al., 1999

[29], Gamoh et al., 1999) [35]. In the SO muscles of the ARA-fed rats, the ratio of DHA/ARA was the lowest, thus suggesting an increased oxidative stress in the SO muscles of the ARA-fed rats. The results were consistent with the highest levels of TBARS in the SO muscles of the ARA-fed rats than the corresponding levels of TBARS in the SO muscles of the DHA-fed and control fibers (the TBARS increased by 19% in the DHA-fed SO muscles and ~30% in the control SO muscles, when compared with those of the ARA-fed SO muscles). There are numerous reports on the fact that: the greater the unsaturation index, the higher the lipid peroxidation is. The value of unsaturation index (USI) was the highest in the SO of the DHA-fed rats, thus one might have expected that DHA, as being one of the members of the highly polyunsaturated fatty acids, would make the SO tissues more vulnerable to the oxidative stress. However, the scenario was quite different in the SO fibers of the DHA-fed rats, as indicated by the lowest values of the TBRAS in these rats. This suggests that DHA prompted an anti-oxidative defense in the SO fibers of DHA-fed rats, otherwise the levels of TBARS could have increased rather it decreased. The inference is consistent with the increased mRNA levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD) as compared to the mRNA levels in SO of the ARA-fed rats. The mRNA levels of catalase (CAT), however, was lower in DHA-fed SO than that of the ARA-fed SO fibers. Compared with the controls, the mRNA levels of SOD also were not changed, thus has remained without further predication.

The oral administration of DHA- and ARA-oils to the rats resulted in a little bit different scenario in the EDL muscles of the rats. Despite of having a significant difference in the values of unsaturation index, n6/n3 or DHA/AA molar ratios among the EDL fibers of controls, DHA-fed and ARA-fed rats, the levels of TBARS, however, were not altered at all. The results thus suggest that the DHA and ARA play a differential role in the slow-twitch and fast-twitch muscles. The mRNA level of catalase increased in the EDL of the DHA-fed rats

than that of the EDL of the ARA-fed rats, while the opposite was seen in the SO fibers, where mRNA level of the DHA-fed rats rather increased. The mRNA levels of GPx or SOD increased in the DHA-fed rats, when compared with those in the EDL of the ARA-fed rats. The effect of DHA vs. ARA was similar on the GPx and SOD both in the SO and EDL fibers. Histological analysis also revealed a differential effect of DHA and ARA on the muscle fibers. The muscle cell areas per muscle area in the SO were not affected in each group, whereas those in the EDL were decreased in the ARA group (Fig 2H). Ratio of red muscle cell number per muscle cell number in the SO was significantly lower in the DHA group, whereas red and intermediate muscle cell mean areas per muscle cell mean areas was significantly lower in the ARA group (Fig 3G,3H). Furthermore, MHC1, MHC2A and MHC2B expressions in the SO were significantly decreased in the DHA group. MHC1 and MHC2A expressions in the EDL were significantly higher in the DHA group, whereas these were significantly lower in the ARA group. Thus, our study indicates a dietary fatty acid-dependent, differential regulation of contractile and metabolic properties between slow-type and fast-type muscles. There was no influence on body weight suggesting that there was no general systemic toxicity caused by chronic administrations of either DHA- or ARA-oil. The fatty acid composition of skeletal muscle varies by muscle fiber type [36-39]. It could be reflected in the content of sarcolemma, sarcoplasmic reticulum, and mitochondria, which have different lipid composition in their membranes [40]. DHA level in the skeletal muscle may affect the muscle functions *in vitro* [41] and *in vivo* [42]. Stark et al. (2007) 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 [43].

The levels of linoleic acid (LA) in the DHA and 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. ARA administration significantly increased the plasma levels of ARA, prostaglandin (PG) E2,

and PGD2. However, DHA administration did not produce any different effects compared with those in the control rats. Furthermore, the inflammatory cytokine levels were not affected by the administration of ARA or DHA.

Multiple damages may explain the decrease in respiration rate and activities of different respiratory chain complexes in human skeletal muscles [44, 45]. Low-level reactive oxygen species plays an important role as the signal transducer of the excitation–contraction coupling; however, high-concentration oxygen species acts to decrease the function of skeletal muscle [46, 47]. Furthermore, oxidative stress is increased in the muscles of elderly people in association with cellular lipid, protein, and DNA damage. In the present study, strongly significant negative correlations were observed between plasma TBARS levels and DHA levels, the DHA/ARA and the EPA/ARA ratios in the EDL. Furthermore, the relative mRNA expressions of glutathione peroxidase, catalase and superoxide dismutase significantly decreased in EDL of ARA group. In previous research, we reported that chronic ARA administration induces muscle cell volume loss with an increase in oxidative stress in aged rats. Thereby, these results also suggest that chronic ARA administration might induce muscle cell volume loss of the EDL with an increase in oxidative stress in young rats. Furthermore, fish oil containing high n-3 PUFA, including EPA and DHA have been reported to improve lipid metabolism. In humans, the oxidation of lipids is increased by the consumption of dietary fish oil [46], which suggests that n-3 PUFA can reduce adiposity. Additionally, the relative mRNA expressions of catalase significantly decreased in SO of DHA group, whereas the relative mRNA expressions of glutathione peroxidase significantly increased in SO of DHA group.

Skeletal muscle has different fiber-types, which are characterized by the expression of distinct myosin variants. MHC1 is expressed in type 1 muscle fibers. Type 2 fibers are further subdivided into type 2A, 2X, and 2B muscle fibers, in which MHC2A, MHC2X, and

MHC2B are preferentially expressed, respectively. Type MHC2A and MHC2X fibers have intermediate characteristics between type 1 (MHC1) and type MHC2B fibers. Although type MHC2X fibers are sometimes defined as fast-twitch glycolytic fibers, type MHC2B fibers have an even stronger fast-twitch glycolytic phenotype than type MHC2X fibers [49-51]. Many aspects of muscle metabolism are believed to correspond to MHC isoform composition. The biological significance between type MHC2X and type MHC2B fibers has not been fully elucidated. However, a previous study reported that the activity of succinate dehydrogenase, a metabolic enzyme involved in the citric acid cycle, was higher in the intermediate type MHC2X fibers than in the fastest type MHC2B fibers [53].

Muscle aging determines the decline in cross-sectional area, fiber denervation, and fiber number loss, primarily type 2 fibers [52, 53]. mRNA abundance in MHC2A and MHC2X fibers was decreased in vastus lateralis biopsy samples from healthy older individuals compared with those from the healthy younger individuals, whereas the MHC1 mRNA content remained unchanged [54]. In contrast, there is no difference between younger and older individuals in terms of mRNA abundance in MHC isoforms [55]. In present study, MHC1, MHC2A and MHC2B expressions in the SO were significantly decreased in the DHA group. MHC1 and MHC2A expressions in the EDL were significantly higher in the DHA group, whereas these were significantly lower in the ARA group. Furthermore, ratio of red muscle cell number per muscle cell number in the SO was significantly lower in the DHA group, whereas red and intermediate muscle cell mean areas per muscle cell mean areas was significantly lower in the ARA group.

Conclusions

In conclusion, the effects of fatty acid on MHC composition and expression of oxidative metabolism-related genes was not in agreement between slow-twitch muscles and fast-twitch muscles. The intake of different types of fatty acids showed an evident effect on rat skeletal muscle contractile and metabolic gene expressions in the SO and EDL. In particular, ARA oil intake showed more oxidative characteristics than DHA oil intake in the plasma and slow-twitch muscles. Further studies are required to clarify the effects of different types of fatty acids on various muscle functions, and to unravel the mechanism underlying the action of slow-type and fast-type muscles. Thus, our study indicates a dietary fat-/fatty acid-dependent, differential regulation of contractile and metabolic properties between slow-type and fast-type muscles.

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Conflicts of interest

The authors declares ‘no conflict of interest’

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Legends of Figures

Fig 1. The relative mRNA expressions of antioxidant enzymes in the skeletal muscles. SO: slow-twitch muscle; EDL: fast-twitch muscle. Means ± SE (n = 8). * = significantly different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). NS = Not significant

Fig 2. Effect of DHA versus ARA on muscle cell areas. SO: slow-twitch muscle; EDL: Fast-twitch muscle. (A-C): The cross-section of SO in each group. (D-F): The cross-section of EDL in each group. (G): The muscle area of the SO and EDL in each group. (H): The muscle cell area per muscle area of SO and EDL in each group. Muscle areas, as measured by cross-sectional areas, were not affected by either DHA- or ARA-oil treatment in both the SO and EDL group (Fig. 2G). The muscle cells per area (muscle area) significantly reduced in the ARA-treated EDL muscle when compared to those of the control or DHA-treated muscles. Again, the muscle cells per area were not affected by either DHA or ARA treatment in the SO muscles. * = significantly different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). NS = Not significant. Scale = 200 μm.

Fig 3. Histological changes of muscle cell type. SO: slow-twitch muscle; EDL: Fast-twitch muscle. (A-C): The cross-section of SO in each group. (D-F): The cross-section of EDL in each group. (G): The ratio of number of red muscle cells per muscle cells of the SO in each group. (H): The red and intermediate muscle cell mean areas per muscle cell mean areas of EDL in each group. Means ± SE (n = 8). * = significantly different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). NS = Not significant.

Fig 4. Effect of oral administration of DHA and ARA-oil on the mRNA levels of myosin heavy chain isoforms (MHC) in slow- and fast-twitch muscles. SO, slow-twitch muscle; EDL, fast-twitch muscle.

(A): Scanned image of electrophoretic gel separation of MHC isoforms and MHC isoform

ratios in the SO and EDL of each group. (B): The relative mRNA expressions of MHC isoforms in the SO and EDL of each group. Means ± SE (n = 8). * = significantly different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). NS = Not significant.

Table 1

Table 1. Fatty acid **c**omposition (mol%) of control-, DHA- and ARA-oil

	Control oil	DHA oil	ARA oil
Palmitic acid, C16:0	13.8 ± 0.01	29.8 ± 0.03	6.95 ± 0.00
Stearic acid, C18:0	13.8 ± 0.01	8.10 ± 0.04	5.91 ± 0.00
Oleic acid, C18:1n-9	42.5 ± 0.03	16.3 ± 0.01	5.31 ± 0.00
Linoleic acid, C18:2 n-6	20.0 ± 0.02	1.96 ± 0.01	9.38 ± 0.01
Arachidonic acid, C20:4n-6 (ARA)	ND	2.49 ± 0.02	45.1 ± 0.04
Eicosapentaenoic acid, C20:5n-3 (EPA)	0.13 ± 0.01	6.61 ± 0.00	0.52 ± 0.00
Docosapentaenoic acid, C22:5n-3	ND	1.17 ± 0.01	ND
Docosahexaenoic acid, C22:6n-3 (DHA)	ND	32.6 ± 0.03	ND

Results are mean ± standard error or mean (SE), each with triplicate determinations. N.D. Not detected.

Table 2

Table 2. List of primers for real-time PCR

Gene	Forward primer	Reverse primer
MHCI	GAATGGCAAGACGGTGACTGT	GGAAGCGTACCTCTCCTTGAGA
MHC2A	ATGACAACTCCTCTCGCT TTGG	TTAAGCTGGAAAGTGACCCGG
MHC2X	CCAATGAGACTAAGACGCCTGG	GCTATCGATGAATTGTCCCTCG
MHCB	GAACACGAAGCGTGTCATCCA	AGGTTTCGATATCTGCGGAGG
GPx	GGAGAATGGCAAGAATGAAGA	CCGCAGGAAGGTAAAGAG
CAT	ATGAAGCAGTGGAAGGAGCA	TCAAAGTGTGCCATCTCGTC
SOD	ATCTTCTTGTGCAGTGCCAGC	CCTTGACTGTGCCGTTGAACT
GAPDH	TGCCGCCTGGAGAAACCT GC	TGAGAGCAATGCCAGCCCCA

MHC, myosin heavy chain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase, GPx, Glutathione peroxidase, CAT = Catalase, SOD = Superoxide dismutase.

Table 3

Table 3. Fatty acid profiles (mol%) and oxidative status in plasma

	Control (n=8)	DHA(n=8)	ARA(n=8)
Palmitic acid, C16:0	24.4±0.25^{ab}	25.14±0.29^{a}	23.48±0.40^{b}
Stearic acid, C18:0	11.2±0.33^{a}	11.43±0.30^{a}	11.90±0.29^{a}
Oleic acid, C18:1n-9	13.7±0.58^{a}	10.97±0.38^{b}	10.04±0.66^{b}
Linoleic acid, C18:2 n-6	19.2±0.63^{a}	19.07±0.49^{a}	14.96±0.81^{b}
Arachidonic acid, C20:4n-6 (ARA)	27.3±0.91^{a}	21.95±0.56^{b}	36.40±1.46^{c}
Eicosapentaenoic acid, C20:5n-3 (EPA)	0.22±0.02^{a}	2.34±0.23^{b}	0.09±0.01^{a}
Docosapentaenoic acid, C22:5n-3	0.40±0.02^{a}	0.72±0.07^{b}	0.36±0.01^{a}
Docosahexaenoic acid, C22:6n-3 (DHA)	2.20±0.15^{a}	7.24±0.25^{b}	1.52±0.07^{c}
n-6/n-3	13.9±0.72^{a}	3.88±0.22^{b}	21.61±0.71^{c}
DHA/ARA	0.08±0.01^{a}	0.33±0.02^{b}	0.04±0.00^{a}
EPA/ARA	0.01±0.01^{a}	0.11±0.01^{b}	0.01±0.01^{a}
SCD index (OLA/STA)	1.24±0.08^{a}	0.97±0.05^{b}	0.86±0.08^{b}
Unsaturation index (USI)	180±2.0^{a}	197±1.6^{b}	198±3.9^{b}
TBARS (nmol/mL)	4.2±0.48^{ab}	3.2±0.27^{a}	5.16±0.57^{b}

Values are Means ± SE. ^{a,b,c} Values in the same row for a given parameter sharing common superscripts are not significantly different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). TBARS, thiobarbituric acid reactive substances, which reflect the levels of lipid peroxide (LPO).

Table 4

Table 4. Fatty acid profiles (mol%) and oxidative status in slow-twitch muscle

	Control (n=8)	DHA(n=8)	ARA(n=8)
Palmitic acid, C16:0	20.0±0.59^{a}	23.0±0.65^{b}	24.0±0.78^{b}
Stearic acid, C18:0	18.9±0.57^{a}	15.8±0.83^{b}	15.5±1.09^{b}
Oleic acid, C18:1n-9	14.1±2.32^{a}	15.5±2.54^{ab}	19.2±0.90^{b}
Linoleic acid, C18:2 n-6	22.3±0.27^{a}	21.9±0.82^{a}	20.3±0.93^{a}
Arachidonic acid, C20:4n-6 (ARA)	16.0±0.67^{a}	9.10±0.75^{b}	16.05±1.47^{a}
Eicosapentaenoic acid, C20:5n-3 (EPA)	0.05±0.00^{a}	0.45±0.04^{b}	0.04±0.00^{a}
Docosapentaenoic acid, C22:5n-3	1.40±0.05^{a}	1.13±0.08^{a}	0.82±0.08^{b}
Docosahexaenoic acid, C22:6n-3	6.60±0.29^{a}	12.2±0.71^{b}	3.09±0.35^{c}
n-6/n-3	4.60±0.15^{a}	2.20±0.12^{b}	8.00±0.55^{c}
DHA/ARA	0.40±0.02^{a}	1.40±0.07^{b}	0.20±0.01^{c}
EPA/ARA	0.00±0.0^{a}	0.05±0.0^{b}	0.00±0.0^{a}
SCD index (OLA/STA)	0.75±0.08^{a}	1.04±014^{ab}	1.33±0.19^{b}
Unsaturation index (USI)	171±3.05^{a}	179±4.9^{a}	149±5.0^{b}
TBARS (nmol/mg of protein)	38.8±2.60^{a}	42.5±4.90^{a}	50.0±3.40^{b}

Values of fatty acids are expressed as mol % of total fatty acids. Means ± SE (n = 8). ^{a,b,c}Values in the same row for a given parameter sharing common superscripts are not significantly different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). TBARS, thiobarbituric acid reactive substances (TBARS), which reflect the levels of lipid peroxide (LPO).

Table 5

Table 5. Fatty acid profiles and oxidative status in fast-twitch muscle

	Control (8)	DHA (8)	ARA (8)
Palmitic acid, C16:0	24.0±0.40[a]	24.0±0.9[a]	25.0±0.30[a]
Stearic acid, C18:0	16.9±0.22[ab]	16.0±0.60[a]	18.0±0.25[b]
Oleic acid, C18:1n-9	11.8±0.60[a]	10.1±0.30[a]	10.6±0.56[a]
Linoleic acid, C18:2 n-6	21.5±0.34[a]	20.3±0.44[a]	12.9±0.43[b]
Arachidonic acid, C20:4n-6 (ARA)	15.3±0.39[a]	9.7±0.39[b]	24.5±0.56[c]
Eicosapentaenoic acid, C20:5n-3 (EPA)	0.06±0.00[a]	0.40±0.04[b]	0.04±0.00[a]
Docosapentaenoic acid, C22:5n-3	1.60±0.05[a]	1.20±0.05[b]	1.50±0.05[a]
Docosahexaenoic acid, C22:6n-3(DHA)	8.2±0.21[a]	17.4±0.27[b]	6.5±0.16[c]
n-6/n-3	3.7±0.11[a]	1.6±0.05[b]	4.6±0.07[c]
DHA/ARA	0.5±0.02[a]	1.8±0.09[b]	0.25±0.00[c]
EPA/ARA	0.00±0.0[a]	0.05±0.0[b]	0.00±0.0[a]
SCD index (OLA/STA)	0.70±0.04	0.60±0.04	0.59±0.04
Unsaturation index (USI)	174±1.3[a]	203±1.0[b]	183±2.0[c]
TBARS (nmol/mg of protein)	39.0±3.60[a]	44.0±4.9[a]	40.0±2.9[a]

Values of fatty acids are expressed as mol % of total fatty acids. Means ± SE (n = 8). [a,b,c]Values in the same row for a given parameter sharing common superscripts are not significantly different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). TBARS, thiobarbituric acid reactive substances, which reflect the levels of lipid peroxide (LPO).

Table 6

Table 6. Correlation coefficients between fatty acid in plasma and fatty acid in slow- and fast- twitch muscles

	Slow-twitch muscle (SO)					
X	**Y**					
Plasma fatty acids	LA (mol %)	ARA (mol %)	DHA (mol %)	n6 / n3	DHA/ARA	EPA/ARA
LA (mol%)	N.S.	N.S.	0.60 (0.005)	-0.60 (0.001)	0.47 (0.023)	N.S.
ARA (mol%)	N.S.	0.50 (0.012)	-0.85 (0.000)	0.88 (0.000)	-0.79 (0.000)	-0.70 (0.000)
EPA (mol%)	N.S.	-0.72 (0.000)	0.87 (0.000)	-0.76 (0.000)	0.92 (0.000)	0.97 (0.000)
DHA (mol%)	N.S.	-0.73 (0.000)	0.92 (0.000)	-0.81 (0.000)	0.95 (0.000)	0.96 (0.000)

	Fast-twitch muscle (EDL)					
X	**Y**					
Plasma fatty acids	LA (mol %)	ARA (mol %)	DHA (mol %)	n6 / n3	DHA/ARA	EPA/ARA
LA (mol%)	0.81 (0.000)	-0.69 (0.000)	0.44 (0.029)	-0.49 (0.015)	0.44 (0.034)	N.S.
ARA (mol%)	-0.79 (0.000)	0.91 (0.000)	-0.78 (0.000)	0.81 (0.000)	-0.78 (0.000)	-0.72 (0.000)
EPA (mol%)	N.S.	-0.77 (0.000)	0.94 (0.000)	-0.91 (0.000)	0.93 (0.000)	0.97 (0.000)
DHA (mol%)	0.45 (0.028)	-0.82 (0.000)	0.98 (0.000)	-0.96 (0.000)	0.97 (0.000)	0.95 (0.000)

LA, linoleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; N.S, not significant. Results are evaluated with simple regression analysis. P values are expressed inside the parentheses.

Table 7

Table 7. Correlation coefficients between the oxidative status in plasma and fast-twitch muscles and fatty acid in slow- and fast-twitch muscles

X	Y											
Plasma TBARS [nmol/mL]	Slow-twitch muscle (SO)						Fast-twitch muscle (EDL)					
	LA (mol%)	ARA (mol%)	DHA (mol%)	n6 / n3	DHA/ ARA	EPA/ ARA	LA (mol%)	ARA (mol%)	DHA (mol%)	n6 / n3	DHA/ ARA	EPA/ ARA
	N.S.	N.S.	-0.53	0.48	-0.53	-0.48	N.S.	0.48	-0.52	0.54	-0.49	-0.49
			(0.008)	(0.017)	(0.008)	(0.010)		(0.017)	(0.009)	(0.007)	(0.016)	(0.016)

LA, linoleic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; N.S, not significant; TBARS, thiobarbituric acid reactive substances; Results are evaluated with simple regression analysis. *P* values are expressed inside the parentheses.

Figure 1

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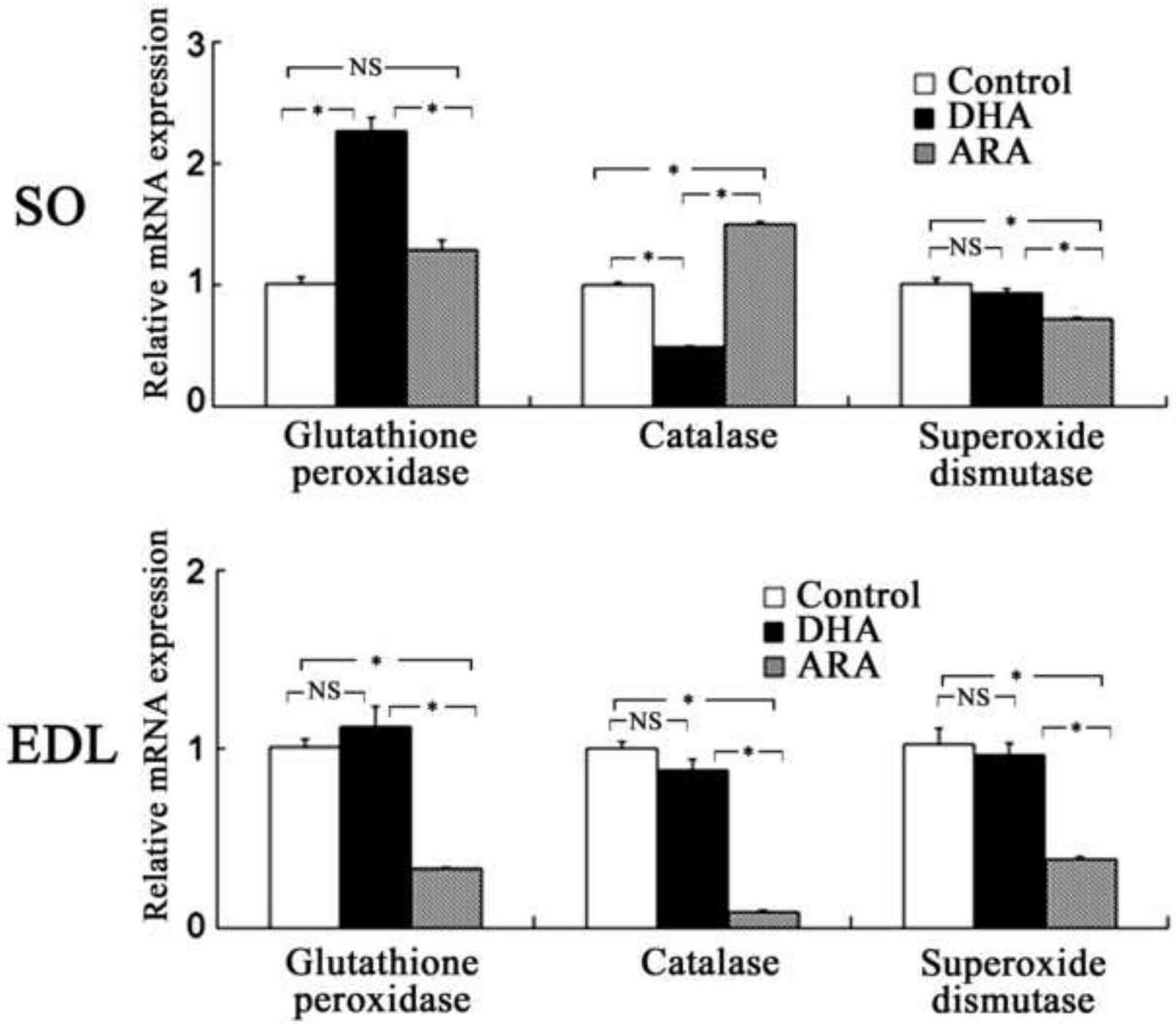

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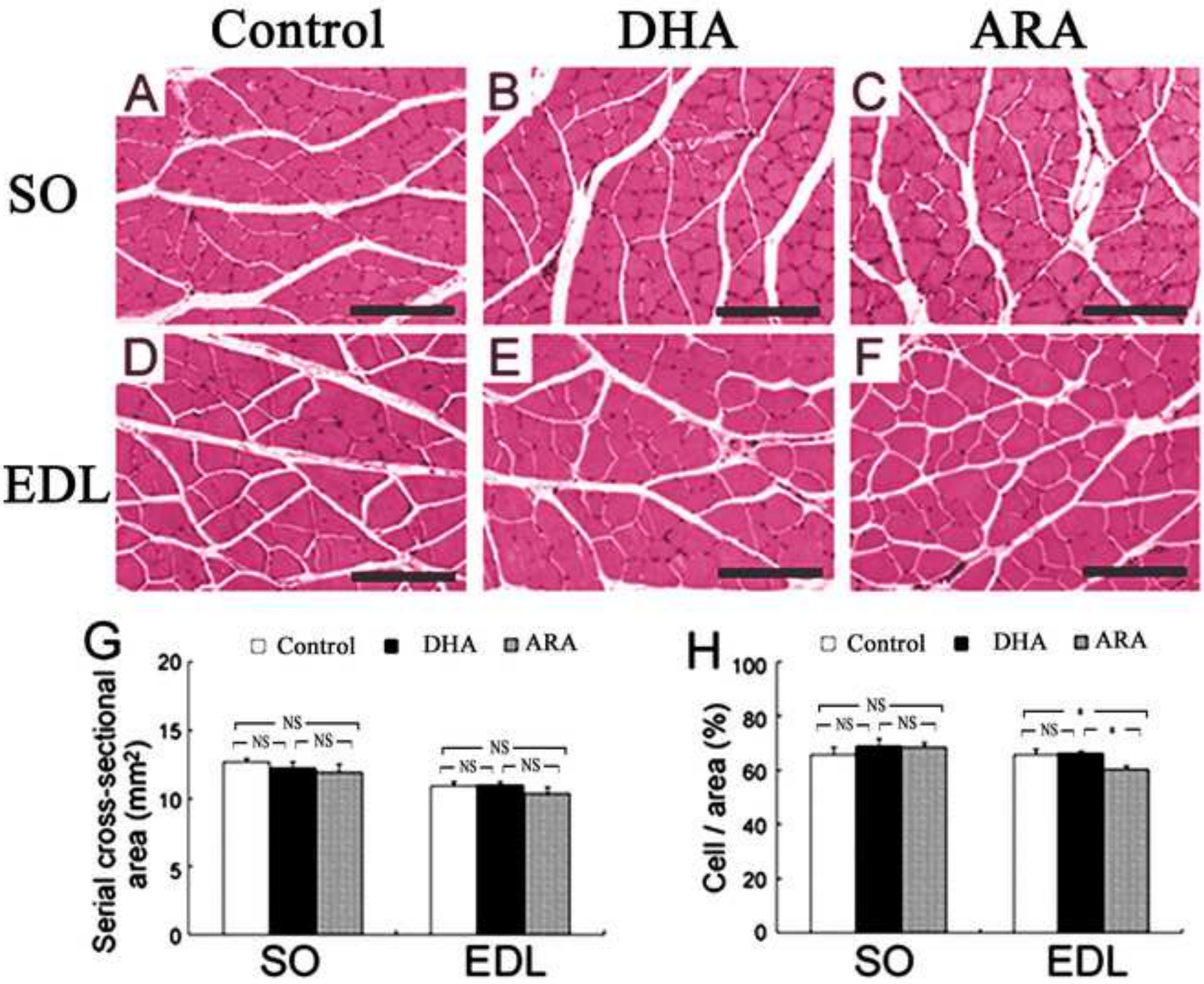

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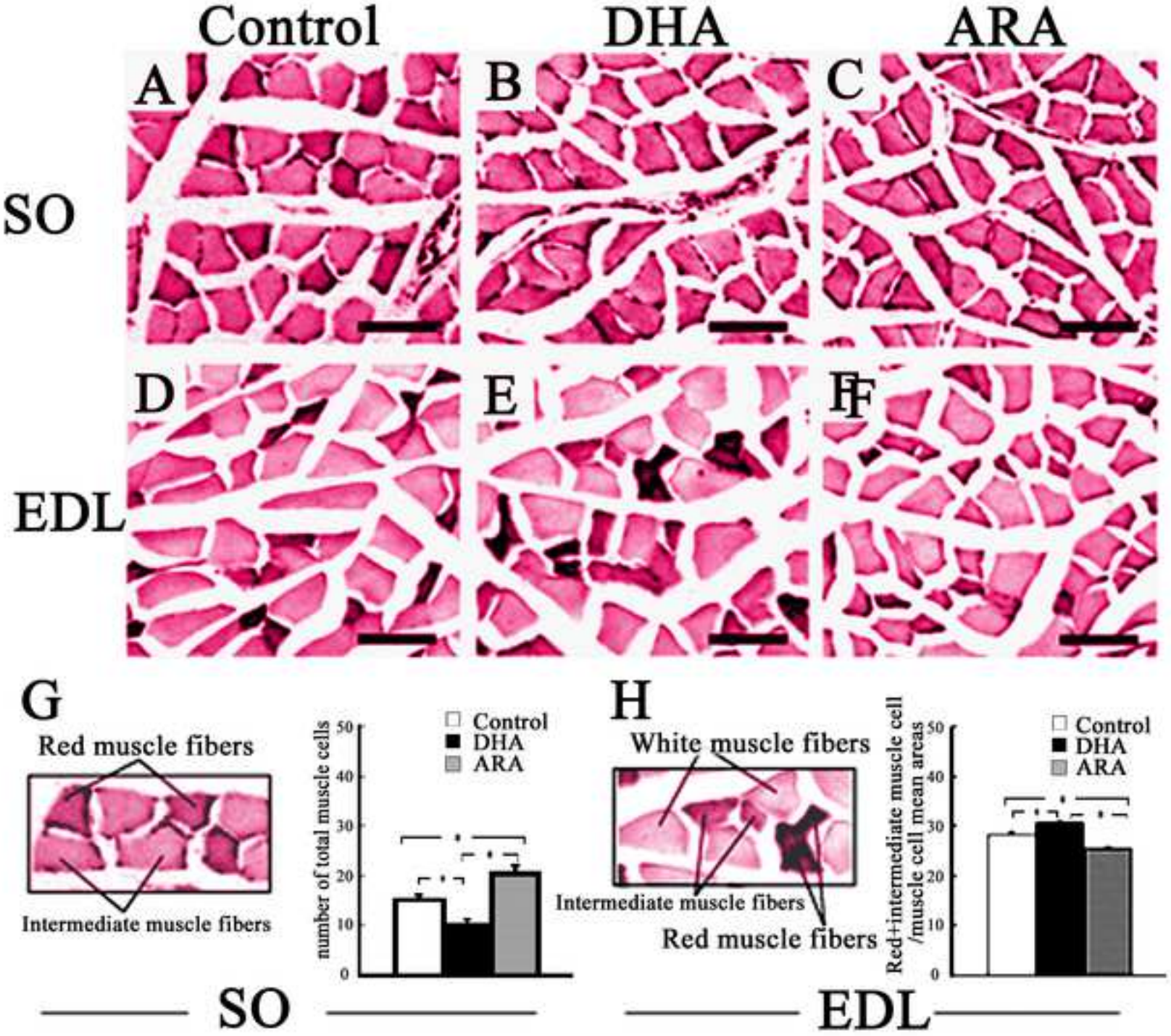

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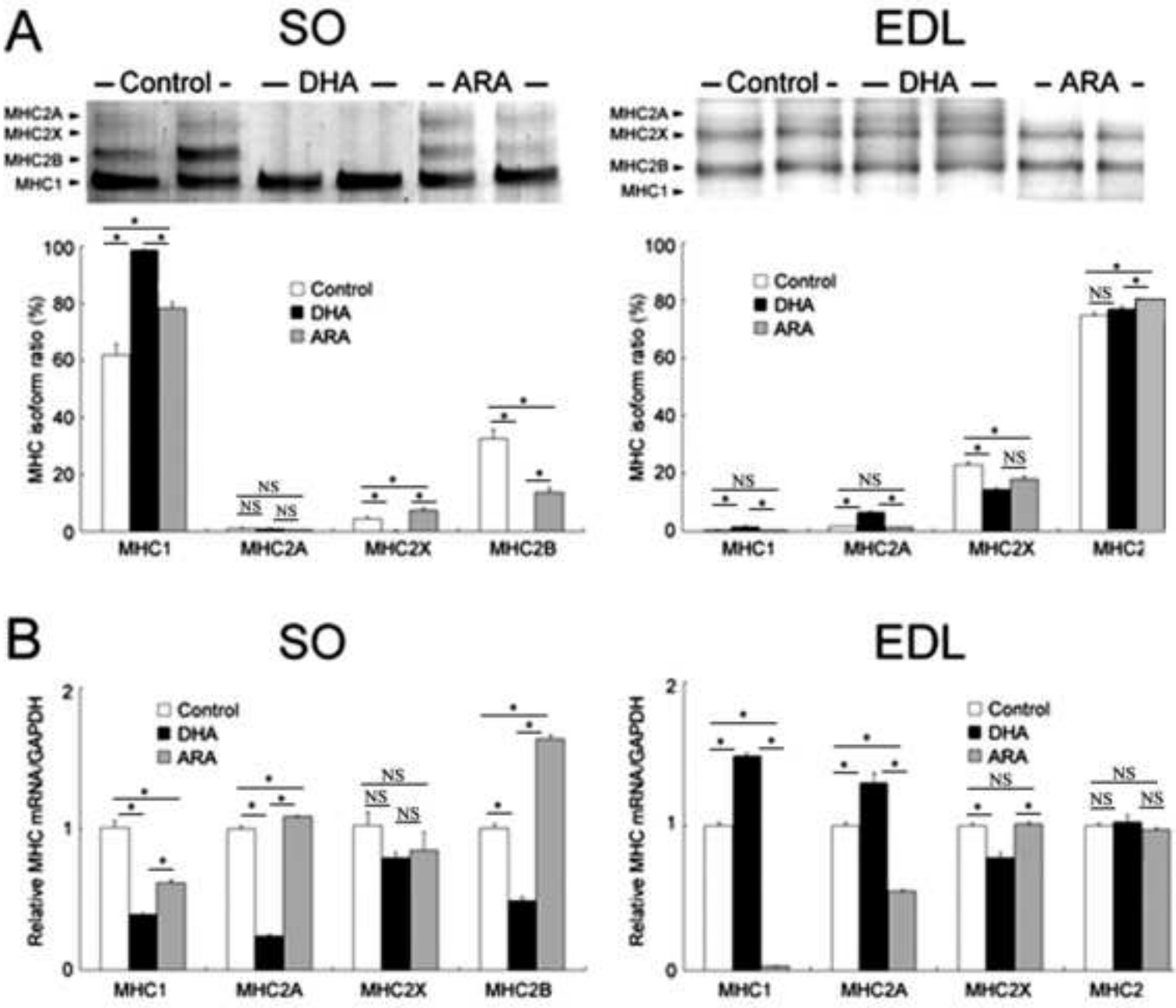

Table 1. Composition of fatty acids in control, DHA, ARA oil.

	Control oil	DHA oil	ARA oil
Palmitic acid C16:0 (mol%)	13.8 ± 0.01	29.8 ± 0.03	6.95 ± 0.00
Stearic acid C18:0 (mol%)	13.8 ± 0.01	8.10 ± 0.04	5.91 ± 0.00
Oleic acid C18:1n-9 (mol%)	42.5 ± 0.03	16.3 ± 0.01	5.31 ± 0.00
Linoleic acid C18:2 n-6 (mol%)	20.0 ± 0.02	1.96 ± 0.01	9.38 ± 0.01
Arachidonic acid C20:4n-6 (ARA) (mol%)	ND	2.49 ± 0.02	45.1 ± 0.04
Eicosapentaenoic acid C20:5n-3 (EPA) (mol%)	0.13 ± 0.01	6.61 ± 0.00	0.52 ± 0.00
Docosapentaenoic acid C22:5n-3 (mol%)	ND	1.17 ± 0.01	ND
Docosahexaenoic acid C22:6n-3 (DHA) (mol%)	ND	32.6 ± 0.03	ND

ND, not detected

Table 2 List of primers for real-time PCR

Gene	Forward primer	Reverse primer	Accession No.
MHCI	GAATGGCAAGACGGTGACTGT	GGAAGCGTACCTCTCCTTGAGA	x 15939
MHC2A	ATGACAACTCCTCTCGCT TTGG	TTAAGCTGGAAAGTGACCCGG	xm_340817
MHC2X	CCAATGAGACTAAGACGCCTGG	GCTATCGATGAATTGTCCCTCG	xm_213345
MHCB	GAACACGAAGCGTGTCATCCA	AGGTTTCGATATCTGCGGAGG	xm_340818
Glutathione peroxidase	GGAGAATGGCAAGAATGAAGA	CCGCAGGAAGGTAAAGAG	
Catalase	ATGAAGCAGTGGAAGGAGCA	TCAAAGTGTGCCATCTCGTC	
Superoxide dismutase	ATCTTCTTGTGCAGTGCCAGC	CCTTGACTGTGCCGTTGAACT	
GAPDH	TGCCGCCTGGAGAAACCT GC	TGAGAGCAATGCCAGCCCCA	M17701

1 MHC, myosin heavy chain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Commented [t1]: Accession No.

Commented [t2]: Accession No.

Commented [t3]: Accession No.

Table 3 Body weight

	Control	DHA	ARA
Number of rats	8	8	8
	417.68±9.30[a]	444.38±5.52[a]	414.61±10.33[a]

ARA, arachidonic acid; DHA, docosahexaenoic acid

Table 4 Fatty acid profiles and oxidative status in plasma

	Control	DHA	ARA
Number of rats	8	8	8
Palmitic acid C16:0 (mol%)	24.44±0.25^{ab}	25.14±0.29^{a}	23.48±0.40^{b}
Stearic acid C18:0 (mol%)	11.22±0.33^{a}	11.43±0.30^{a}	11.90±0.29^{a}
Oleic acid C18:1n-9 (mol%)	13.74±0.58^{a}	10.97±0.38^{b}	10.04±0.66^{b}
Linoleic acid C18:2 n-6 (mol%)	19.18±0.63^{a}	19.07±0.49^{a}	14.96±0.81^{b}
Arachidonic acid C20:4n-6 (ARA) (mol%)	27.29±0.91^{a}	21.95±0.56^{b}	36.40±1.46^{c}
Eicosapentaenoic acid C20:5n-3 (EPA) (mol%)	0.22±0.02^{a}	2.34±0.23^{b}	0.09±0.01^{a}
Docosapentaenoic acid C22:5n-3 (mol%)	0.38±0.02^{a}	0.72±0.07^{b}	0.36±0.01^{a}
Docosahexaenoic acid C22:6n-3 (DHA) (mol%)	2.20±0.15^{a}	7.24±0.25^{b}	1.52±0.07^{c}
n-6/n-3	13.89±0.72^{a}	3.88±0.22^{b}	21.61±0.71^{c}
DHA/ARA	0.08±0.01^{a}	0.33±0.02^{b}	0.04±0.00^{a}
EPA/ARA	0.01±0.01^{a}	0.11±0.01^{b}	0.01±0.01^{a}
SCD index (OLA/STA)	1.24±0.08^{a}	0.97±0.05^{b}	0.86±0.08^{b}
Unsaturation index (USI)	179.53±2.04^{a}	197.30±1.60^{b}	198.34±3.90^{b}
TBARS (nmol/mL)	4.23±0.48^{ab}	3.15±0.27^{a}	5.16±0.57^{b}

Values of fatty acids are expressed as mol % of total fatty acids. Means ± SE (n = 8). a,b,cValues in the same row for a given parameter sharing common superscripts are not significant different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). TBARS, thiobarbituric acid reactive substances

Table 5 Fatty acid profiles and oxidative status in slow twitch muscle

	Control	DHA	ARA
Number of rats	8	8	8
Palmitic acid C16:0 (mol%)	20.02±0.59[a]	23.07±0.65[b]	24.00±0.78[b]
Stearic acid C18:0 (mol%)	18.87±0.57[a]	15.79±0.83[b]	15.51±1.09[b]
Oleic acid C18:1n-9 (mol%)	14.14±2.32[a]	15.53±2.54[ab]	19.20±0.91[b]
Linoleic acid C18:2 n-6 (mol%)	22.33±0.27[a]	21.94±0.82[a]	20.30±0.93[a]
Arachidonic acid C20:4n-6 (ARA) (mol%)	16.04±0.67[a]	9.14±0.75[b]	16.06±1.47[a]
Eicosapentaenoic acid C20:5n-3 (EPA) (mol%)	0.05±0.00[a]	0.45±0.04[b]	0.04±0.00[a]
Docosapentaenoic acid C22:5n-3 (mol%)	1.38±0.05[a]	1.13±0.08[a]	0.82±0.08[b]
Docosahexaenoic acid C22:6n-3 (DHA) (mol%)	6.57±0.29[a]	12.21±0.71[b]	3.09±0.35[c]
n-6/n-3	4.62±0.15[a]	2.22±0.12[b]	8.04±0.55[c]
DHA/ARA	0.41±0.02[a]	1.37±0.07[b]	0.19±0.01[c]
EPA/ARA	0.00±0.0[a]	0.05±0.0[b]	0.00±0.0[a]
SCD index (OLA/STA)	0.76±0.08[a]	1.04±014[ab]	1.33±0.19[b]
Unsaturation index (USI)	170.70±3.06[a]	178.69±4.89[a]	149.14±5.03[b]
TBARS (nmol/mg of protein)	38.77±2.59[a]	42.49±4.93[a]	50.35±3.40[a]
ROS (nmol/min/mg of protein)	2.07±0.13[a]	5.21±0.56[b]	1.63±0.07[a]

Values of fatty acids are expressed as mol % of total fatty acids. Means ± SE (n = 8). a,b,cValues in the same row for a given parameter sharing common superscripts are not significant different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). TBARS, thiobarbituric acid reactive substances; ROS, reactive oxygen species.

Table 6 Fatty acid profiles and oxidative status in fast twitch muscle

	Control	DHA	ARA
Number of rats	8	8	8
Palmitic acid C16:0 (mol%)	24.16±0.44[a]	24.22±0.85[a]	25.21±0.30[a]
Stearic acid C18:0 (mol%)	16.86±0.22[ab]	16.14±0.61[a]	18.23±0.25[b]
Oleic acid C18:1n-9 (mol%)	11.838±0.60[a]	10.14±0.30[a]	10.62±0.56[a]
Linoleic acid C18:2 n-6 (mol%)	21.54±0.34[a]	20.28±0.44[a]	12.93±0.43[b]
Arachidonic acid C20:4n-6 (ARA) (mol%)	15.30±0.39[a]	9.66±0.39[b]	24.47±0.56[c]
Eicosapentaenoic acid C20:5n-3 (EPA) (mol%)	0.06±0.00[a]	0.43±0.04[b]	0.04±0.00[a]
Docosapentaenoic acid C22:5n-3 (mol%)	1.59±0.05[a]	1.17±0.05[b]	1.51±0.05[a]
Docosahexaenoic acid C22:6n-3 (DHA) (mol%)	8.19±0.21[a]	17.41±0.27[b]	6.47±0.16[c]
n-6/n-3	3.67±0.11[a]	1.56±0.05[b]	4.56±0.07[c]
DHA/ARA	0.54±0.02[a]	1.83±0.09[b]	0.26±0.00[c]
EPA/ARA	0.00±0.0[a]	0.05±0.0[b]	0.00±0.0[a]
SCD index (OLA/STA)	0.70±0.04	0.64±0.04	0.59±0.04
Unsaturation index (USI)	174.34±1.3[a]	202.56±1.0[b]	181.61±2.3[c]
TBARS (nmol/mg of protein)	38.68±3.61[a]	43.99±4.88[a]	40.02±2.93[a]
ROS (nmol/min/mg of protein)	2.55±0.22[a]	2.47±0.19[a]	7.68±0.54[b]

Values of fatty acids are expressed as mol % of total fatty acids. Means ± SE (n = 8). a,b,cValues in the same row for a given parameter sharing common superscripts are not significant different at $P < 0.05$ (one-way ANOVA with Scheffe's *post hoc* test). TBARS, thiobarbituric acid reactive substances; ROS, reactive oxygen species.

Supplemental Table 1　Correlation coefficients between fatty acid in plasma and fatty acid in slow- and fast- twich muscles

Plasma fatty acids	SO					
	LA (mol %)	ARA (mol %)	DHA (mol %)	n6/n3	DHA/ARA	EPA/ARA
LA (mol%)	N.S.	N.S.	0.557 (0.005)	-0.643 (0.001)	0.461 (0.023)	N.S.
ARA (mol%)	N.S.	0.505 (0.012)	-0.854 (0.000)	0.880 (0.000)	-0.786 (0.000)	-0.697 (0.000)
EPA (mol%)	N.S.	-0.718 (0.000)	0.870 (0.000)	-0.755 (0.000)	0.920 (0.000)	0.969 (0.000)
DHA (mol%)	N.S.	-0.734 (0.000)	0.923 (0.000)	-0.811 (0.000)	0.953 (0.000)	0.955 (0.000)

Plasma fatty acids	EDL					
	LA (mol %)	ARA (mol %)	DHA (mol %)	n6/n3	DHA/ARA	EPA/ARA
LA (mol%)	0.809 (0.000)	-0.678 (0.000)	0.445 (0.029)	-0.491 (0.015)	0.435 (0.034)	N.S.
ARA (mol%)	-0.792 (0.000)	0.910 (0.000)	-0.776 (0.000)	0.814 (0.000)	-0.783 (0.000)	-0.719 (0.000)
EPA (mol%)	N.S.	-0.770 (0.000)	0.938 (0.000)	-0.914 (0.000)	0.933 (0.000)	0.967 (0.000)
DHA (mol%)	0.447 (0.028)	-0.824 (0.000)	0.978 (0.000)	-0.955 (0.000)	0.971 (0.000)	0.950 (0.000)

LA, linoleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; N.S, not significance; SO, slow twitch muscle; EDL, fast twitch muscle. Results are evaluated with simple regression analysis. *P* values are expressed inside the parentheses.

Supplemental Table 2 Correlation coefficients between the oxidative status in plasma and fast-twitch muscles and fatty acid in slow- and fast-twitch muscles

	SO					
	LA (mol %)	ARA (mol %)	DHA (mol %)	n6/n3	DHA/ARA	EPA/ARA
Plasma TBARS (nmol/mL)	N.S.	N.S.	-0.531 (0.008)	0.481 (0.017)	-0.531 (0.008)	-0.477 (0.010)
SO ROS (nmol/min/mg of protein)	N.S.	-0.642 (0.001)	0.814 (0.000)	-0.718 (0.000)	0.870 (0.000)	0.851 (0.000)

	EDL					
	LA (mol %)	ARA (mol %)	DHA (mol %)	n6/n3	DHA/ARA	EPA/ARA
Plasma TBARS (nmol/mL)	N.S.	0.484 (0.017)	-0.524 (0.009)	0.536 (0.007)	-0.487 (0.016)	-0.487 (0.016)
EDL ROS (nmol/min/mg of protein)	-0.866 (0.000)	0.851 (0.000)	-0.590 (0.002)	0.686 (0.000)	-0.600 (0.002)	-0.526 (0.008)

LA, linoleic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; N.S, not significance; TBARS, thiobarbituric acid reactive substances; ROS, reactive oxygen species; SO, slow twitch muscle; EDL, fast twitch muscle. Results are evaluated with simple regression analysis. *P* values are expressed inside the parentheses.