Prostaglandins, Leukotrienes and Essential Fatty Acids 91 (2014) 119–127

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

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

Original Article

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

Takayuki Inoue [a,b,1,2], Michio Hashimoto [a,*], Masanori Katakura [a,1], Yoko Tanabe [a,1], Abdullah Al Mamun [a,1], Kentaro Matsuzaki [a,1], Hiroki Otani [b,2], Osamu Shido [a,1]

[a] Department of Environmental Physiology, Faculty of Medicine, Shimane University, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan
[b] Department of Developmental Biology, Faculty of Medicine, Shimane University, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan

ARTICLE INFO

Article history:
Received 14 March 2014
Received in revised form
19 July 2014
Accepted 21 July 2014

Keywords:
n-6 fatty acid
Oxidative stress
Aging
Skeletal muscles
Myosin heavy chain

ABSTRACT

Arachidonic acid (20:4*n*−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 (20:4*n*−6, ARA), a polyunsaturated fatty acid (PUFA) synthesized from linoleic acid (18:2*n*−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

☆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, Culture, Science, Sports and Technology of Japan (#23500955, MH).
* Corresponding author. Tel.: +81 853 20 2112; fax: +81 853 20 110.
E-mail address: michio1@med.shimane-u.ac.jp (M. Hashimoto).
[1] Tel: +81 853 20 2112, fax: +81 853 20 2110.
[2] Tel: +81 853 20 2102, fax: +81 853 20 2100.

http://dx.doi.org/10.1016/j.plefa.2014.07.015
0952-3278/

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 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.

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 C18:1$n-9$	42.5 ± 0.03	5.31 ± 0.00
Linoleic acid C18:2$n-6$	20.0 ± 0.02	9.38 ± 0.01
Arachidonic acid C20:4$n-6$	ND	45.1 ± 0.04
Eicosapentaenoic acid C20:5$n-3$	0.13 ± 0.01	0.52 ± 0.00
Docosapentaenoic acid C22:5$n-3$	ND	ND
Docosahexaenoic acid C22:6$n-3$	ND	ND

ND: Not detected.

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 was 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