Experimental Gerontology 102 (2018) 84–92

Contents lists available at ScienceDirect

Experimental Gerontology

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

Age-related reduction in single muscle fiber calcium sensitivity is associated with decreased muscle power in men and women

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ARTICLE INFO

Keywords:
Myosin heavy chain isoforms
Human
Specific force
Muscle function

ABSTRACT

Age-related declines in human skeletal muscle performance may be caused, in part, by decreased responsivity of muscle fibers to calcium (Ca^{2+}). This study examined the contractile properties of single vastus lateralis muscle fibers with various myosin heavy chain (MHC) isoforms (I, I/IIA, IIA and IIAX) across a range of Ca^{2+} concentrations in 11 young (24.1 ± 1.1 years) and 10 older (68.8 ± 0.8 years) men and women. The normalized pCa-force curve shifted rightward with age, leading to decreased activation threshold (pCa_{10}) and/or Ca^{2+} sensitivity (pCa_{50}) for all MHC isoforms examined. In older adults, the slope of the pCa-force curve was unchanged in MHC I-containing fibers (I, I/IIA), but was steeper in MHC II-containing fibers (IIA, IIAX), indicating greater cooperativity compared to young adults. At sub-maximal [Ca^{2+}], specific force was reduced in MHC I-containing fibers, but was minimally decreased in MHC IIA fibers as older adults produced greater specific forces at high [Ca^{2+}] in these fibers. Lessor pCa_{50} in MHC I fibers independently predicted reduced isokinetic knee extensor power across a range of contractile velocities, suggesting that the Ca^{2+} response of slow-twitch fibers contributes to whole muscle dysfunction. Our findings show that aging attenuates Ca^{2+} responsiveness across fiber types and that these cellular alterations may lead to age-related reductions in whole muscle power output.

1. Introduction

Physical function declines with age in humans (Fielding et al., 2011). Whole muscle contractile performance is a critical determinant of physical function (Bean et al., 2002; Foldvari et al., 2000; Suzuki et al., 2001; Skelton et al., 1994), with both muscle strength and power declining precipitously with advancing age (Delmonico et al., 2009; Clark et al., 2013; Bouchard et al., 2011). Muscle size decreases with age, but atrophy alone does not explain decrements in whole muscle strength and power (Delmonico et al., 2009; Hughes et al., 2001). Whole muscle function is determined, in part, by the intrinsic contractile properties of the underlying muscle fibers (Miller et al., 2014; Bottinelli and Reggiani, 2000; Cormie et al., 2011). Thus, understanding age-related changes at the cellular level is crucial for developing targeted countermeasures to age-related muscle weakness that seek to improve physical function.

The role of calcium (Ca^{2+}) in regulating skeletal muscle contraction is well-known (Gordon et al., 2000; MacIntosh, 2003). However, only a small number of studies have examined the influence of age on the Ca^{2+} response of single skeletal muscle fibers in humans. A recent study found that older adults had a reduction of pCa_{50} in all myosin heavy chain (MHC) II fibers combined (e.g., MHC IIA and IIAX), but did not observe age-related differences in the Ca^{2+} response of MHC I fibers (Lamboley et al., 2015). Two other studies from a different research group showed no significant age-related differences in pCa_{10}, pCa_{50} or slope of the pCa-force curve (Hill coefficient; *h*) in either MHC I or IIA fibers (Hvid et al., 2011; Hvid et al., 2013). While these studies provide valuable insights, several factors that modify age-related single fiber function need to be considered. First, experimental control for physical activity level is important, as physical inactivity and exercise can modulate Ca^{2+} sensitivity (Widrick et al., 1985; Mounier et al., 2009; Yamashita-Goto et al., 2001; Malisoux et al., 2006; Gejl et al.,

Abbreviations: ANOVA, analysis of variance; Ca^{2+}, calcium; CSA, cross-sectional area; DEXA, dual-energy x-ray absorptiometry; EGTA, ethylene glycol-bis(2-amino-ethylether)-N,N,N′,N′-tetraacetic acid; *h*, Hill coefficient; MHC, myosin heavy chain; n, number of fibers; N, number of subjects; pCa, $-\log_{10}[Ca^{2+}]$; pCa_{10}, [Ca^{2+}] at which 10% of maximal force is produced (activation threshold); pCa_{50}, [Ca^{2+}] at which 50% of maximal force is produced (Ca^{2+} sensitivity); SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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https://doi.org/10.1016/j.exger.2017.12.007
Received 3 October 2017; Received in revised form 17 November 2017; Accepted 11 December 2017
Available online 13 December 2017
0531-5565/

2016; Godard et al., 2002). Previous studies did match the young and older populations using questionnaires, but self-reporting of physical activity has limitations (Sallis and Saelens, 2000). Second, previous work evaluated fibers at 120% of slack length (Lamboley et al., 2015; Hvid et al., 2011; Hvid et al., 2013), so the sarcomere length was unknown. This is important as Ca^{2+} sensitivity is altered with sarcomere length, due to changes in the proximity of actin to the myosin binding site (MacIntosh, 2003; Endo, 1972; Wang and Fuchs, 2000). Finally, no study has examined the relationship between the Ca^{2+} responsivity of single muscle fibers and in vivo contractile function to determine whether alterations in cellular function scale to the whole muscle level to contribute to age-related functional deficits.

The aim of the present study was to examine the effects of age on the Ca^{2+} response of chemically-skinned, single vastus lateralis muscle fibers (MHC I, I/IIA, IIA and IIAX) from young and older men and women. Notably, sarcomere length under relaxing conditions (pCa 8) was set at 2.65 μm and physical activity of the young and older groups was matched using accelerometry. Furthermore, we aimed to examine the relationship between single fiber Ca^{2+} sensitivity and whole muscle isokinetic power output of the knee extensors. As an exploratory analysis, we examined whether sex interacted with age to alter Ca^{2+} sensitivity in MHC I and IIA fibers, as we have previously observed sex-specific changes in molecular, cellular and whole muscle contractile function among older adults (Miller et al., 2013; Callahan et al., 2014a).

2. Methods

2.1. Participants

Eleven young (5 men, 6 women) and 10 older (3 men, 7 women) adults participated in this study. Young and older adults had habitual physical activity levels measured over 7 days using accelerometry, as previously described (Toth et al., 2010). In order to match young and older adults for physical activity level, we recruited young adults who were minimally active (not engaged in a structured exercise program) and older adults who were moderately active (~30 min of walking on 3–5 days per week). All volunteers had no symptoms or signs of heart disease, hypertension, or diabetes (fasting blood glucose > 112 mg/dl); normal resting electrocardiogram; normal electrocardiogram response to an exercise stress test; normal thyroid function; and normal blood cell counts and blood biochemistry values. Volunteers were excluded if they currently or had participated in a weight loss or exercise training program in the past year, a history of smoking (within 1 yr), unintentional weight loss of > 2.5 kg during the last 3 mo, a body mass index > 30.0 kg/m^2, a hospitalization longer than 3 days in the past 5 yr, an active neoplasm or history of one within the past 5 yr, or were taking/had taken hormone replacement therapy (combined estrogen/progestin for older women and testosterone for older men). Participants taking statins or oral corticosteroids were excluded. Young women taking oral contraceptives were included (n = 4), and all older women were postmenopausal (cessation of menses > 1 yr). Data on clinical characteristics as well as whole muscle and single fiber structure and function in MHC I and IIA fibers from young and older volunteers have been reported previously (Miller et al., 2013; Callahan et al., 2014b), as have relationships between molecular and cellular function in MHC I, I/IIA, IIA and IIAX fibers from young adults (Miller et al., 2015). The present study examines a subset of the entire cohort, and was limited to those volunteers that had pCa-force measurements performed. All participants provided written informed consent prior to enrollment in this study. The protocol was approved by the Committees on Human Research at the University of Vermont.

2.2. Experimental protocol

Eligibility for participation was determined during screening visits, which also included whole muscle strength testing, a treadmill test, and resting and exercising electrocardiograms. At least one week following the screening visit, a percutaneous needle biopsy of the vastus lateralis muscle was performed under lidocaine anesthesia. The majority of tissue was immediately placed into cold (4 °C) dissecting solution and processed for single fiber mechanical assessment. The remaining tissue was frozen in liquid nitrogen and stored at − 80 °C or prepared for electron microscopy or immunohistochemical assessment, the results of which are presented elsewhere (Callahan et al., 2014b).

2.3. Whole body measurements

Body mass was measured using a digital scale (ScaleTronix, Wheaton, IL) and total and regional body composition was assessed via dual-energy X-ray absorptiometry, or DEXA (GE Lunar, Madison, WI). Leg fat-free mass was determined (Heymsfield et al., 1990) and used as a proxy for muscle mass. Maximum isokinetic knee extensor torque of the right leg was measured, after establishing range of motion and gravity correction, using a dynamometer (Humac Norm, CSMi, Stoughton, MA) at five different isokinetic testing velocities (60, 120, 180, 240 and 300°/s) in a randomized fashion. For each testing speed, participants performed 4 repetitions, and each trial was separated by a 2-min rest period. Following each trial, data were reviewed to ensure that participants attained the target isokinetic velocity in at least 3 of the 4 repetitions. The peak power output was calculated during the isovelocity phase of the contraction for each velocity where the target was achieved.

2.4. Muscle tissue processing

Biopsy tissue was immediately placed into cold (4 °C) dissecting solution (in mM: 20 *N,N*-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid (BES), 5 ethylene glycol-bis(2-amino-ethylether)-N,N,N′,N′-tetra-acetic acid (EGTA), 5 MgATP, 1 free Mg^{2+}, 1 dithiothreitol and 0.25 phosphate (P_i)) with an ionic strength of 175 mEq, pH 7.0, and at pCa 8.0 for isolation of single fiber bundles for mechanical measurements. Muscle bundles of approximately 50 fibers were dissected and tied to glass rods at slightly stretched lengths and placed in skinning solution (in mM: 170 potassium propionate, 10 imidazole, 5 EGTA, 2.5 $MgCl_2$, 2.5 Na_2H_2ATP, 0.05 leupeptin and 0.05 antipain at pH 7.0) for 24 h at 4 °C. After skinning, fibers were placed in storage solution (identical to skinning solution, but with 1 mM sodium azide and without leupeptin and antipain) with increasing concentrations of glycerol (10% *v*/v glycerol for 2 h, 25% glycerol v/v for 2 h) until reaching the final storage solution (50% v/v glycerol), in which they were incubated at 4 °C for 18–20 h. Thereafter, fibers were stored at − 20 °C until isolation of single fibers for mechanical measurements, which occurred within 4 weeks of the biopsy.

2.5. Experimental solutions

Constituents of all solutions used during mechanical measurements were calculated using the equations and stability constants according to Godt and Lindley (Godt and Lindley, 1982). Relaxing solution was dissecting solution with 15 mM creatine phosphate and 300 units/ml of creatine phosphokinase. Pre-activating solution was the same as relaxing solution, except at an EGTA concentration of 0.5 mM. Activating solution was the same as relaxing solution, except at pCa 4.5. All solutions were adjusted to proper ionic strength (175 mEq) using sodium methane sulfate.

2.6. Single fiber pCa-force measurements

The Ca^{2+} response of single muscle fibers is routinely determined by exposing a chemically- or mechanically-skinned fiber to a range of [Ca^{2+}] that produce minimum to maximum force and plotting the results as a pCa-force curve, where pCa = $-\log_{10}[Ca^{2+}]$ and force is