Experimental Gerontology 84 (2016) 40–48

Contents lists available at ScienceDirect

Experimental Gerontology

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

Absence of morphological and molecular correlates of sarcopenia in the macaque tongue muscle styloglossus

Alan J. Sokoloff Ph.D. [a,*], Megan Douglas J.D. [a], Jill A. Rahnert Ph.D. [b], Thomas Burkholder Ph.D [b], Kirk A. Easley MApStat [c], Qingwei Luo M.S.C.R. [a]

[a] Department of Physiology, Emory University School of Medicine, Atlanta, GA, United States
[b] School of Applied Physiology, Georgia Institute of Technology, Atlanta, GA, United States
[c] Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA, United States

ARTICLE INFO

Article history:
Received 7 January 2016
Received in revised form 18 August 2016
Accepted 22 August 2016
Available online 24 August 2016

Section Editor: Christiaan Leeuwenburgh

Keywords:
Tongue
Aging
Sarcopenia
Swallowing
Myosin heavy chain
Human
Muscle

ABSTRACT

Introduction: Equivocal decline of tongue muscle performance with age is compatible with resistance of the tongue to sarcopenia, the loss of muscle volume and function that typically occurs with aging. To test this possibility we characterized anatomical and molecular indices of sarcopenia in the macaque tongue muscle styloglossus (SG).

Methods: We quantified myosin heavy chain (MHC), muscle fiber MHC phenotype and size and total and phosphorylated growth- and atrophy-related proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), immunoblot and immunohistochemistry (IHC) in the SG in twenty-four macaque monkeys (*Macaca rhesus*, age range 9 months to 31 years) categorized into Young (<8 years of age), Middle-aged (15–21 years of age) and Old (>22 years of age) groups.

Results: In Young, Middle and Old age groups, by SDS-PAGE MHCI comprised ~1/3 and MHCII ~2/3 of total MHC. MHCI relative frequency was lower and MHCII higher in Middle versus Young ($p = 0.0099$) and Middle versus Old ($p = 0.052$). Relative frequencies of MHC fiber phenotype were not different by age but were different by phenotype (rates 233, 641 and 111 per 1000 fibers for MHCI, MHCII and MHCI-II respectively, $p = 0.03$). Few or no fibers were positive for developmental MHC. Mean cross-sectional area (CSA) was not different among the three age groups for MHCII and MHCI-II; however MHCI fibers tended to be larger in Middle versus Old and Young (mean = 2257 μm^2,1917 μm^2 ($p = 0.05$) and 1704 μm^2 ($p = 0.06$), respectively). For each age group, mean CSA increased across MHC phenotype (lowest mean CSA for MHCI and highest mean CSA for MHCII). Spearman analysis demonstrated age-related increases in total p70 ribosomal protein S6 kinase (P70), phosphorylated $P70^{421/424}$, phosphorylated P38 mitogen-activated protein kinase and muscle atrophy F-Box, a trend to age-related decrease in total extracellular signal-regulated kinase (ERK), and no age-related change in total protein kinase B (Akt/PKB), phosphorylated Akt, phosphorylated ERK, phosphorylated c-Jun N-terminal kinase (JNK46) and phosphorylated $P70^{389}$.

Conclusion: Common anatomical and molecular indices of sarcopenia are absent in our sample of macaque SG. Relative frequencies of MHCII protein and phenotype are preserved with age. Although MAFbx expression increases with age, this is not associated with fiber atrophy, perhaps reflecting compensatory growth signaling by p70. The resistant nature of the styloglossus muscle to sarcopenia may be related to routine activation of tongue muscles in respiration and swallowing and the preservation of hypoglossal motoneuron number with age.

1. Introduction

Swallowing dysfunction (dysphagia) affects >15% of the aging population with negative impact on quality of life including malnutrition and aspiration (Ney et al., 2009). Many muscles lose mass and function with age, i.e. undergo sarcopenia (Mitchell et al., 2012), and sarcopenia of head and neck muscles has been suggested to contribute to age-related swallowing dysfunction (Robbins et al., 2005). Because the tongue generates pressures critical for swallowing, sarcopenia of the

Abbreviations: Akt/PKB, protein kinase B; DAB, Diaminobenzidine; EDL, extensor digitorum longus; ERK, extracellular signal-regulated kinase; GG, genioglossus; IHC, immunohistochemistry; JNK, c-Jun N-terminal kinase; MAFbx, muscle atrophy F-Box; MHC, myosin heavy chain; P38, P38 mitogen-activated protein kinase; P70, p70 ribosomal protein S6 kinase; PL, plantaris; SG, styloglossus; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TA, tibialis anterior; VL, vastus lateralis.

* Corresponding author at: Department of Physiology, Emory University, 615 Michael Street, Atlanta, GA 30322, United States.
E-mail address: Asokolo@emory.edu (A.J. Sokoloff).

http://dx.doi.org/10.1016/j.exger.2016.08.010
0531-5565/

tongue might contribute to age-related dysphagia. A reported decrease with age in tongue "functional reserve", i.e., the ability to produce tongue force beyond routine requirement for swallowing, is compatible with tongue sarcopenia (e.g., Robbins et al. 2005). However, normal and effortful swallow pressures do not decline with age in women (Yeates et al., 2010) and by some measures tongue functional reserve is maintained with age (Steele, 2013) suggesting tongue muscles may be resistant to sarcopenia and that age-related decline in tongue motor performance may be non-myogenic in origin.

Morphological and molecular features of aging differ by muscle, fiber type, species and gender (e.g., Ciciliot et al., 2013; Deschenes et al., 2013), and it is unclear whether tongue muscles are susceptible to sarcopenia. Features common to sarcopenic appendicular muscle, i.e., a shift to slower myosin heavy chain (MHC) isoforms, Type II muscle fiber atrophy and neuromuscular junction dysmorphology, are absent or minimal in rat tongue muscles (Connor et al., 2009; Oliven et al., 2001; Hodges et al., 2004; Rahnert et al., 2011). In humans, tongue muscle fiber size has been reported to increase (Nakayama, 1991) or decrease (Rother et al., 2002) with age. Previously we noted stability of MHCI and MHCIIX relative frequency and a trend to decreased MHCIIA relative frequency in the human tongue muscle genioglossus with age (Daugherty et al., 2012) but failed to find appreciable developmental MHC, indicative of regenerating sarcopenic muscle (Snow et al., 2005), even in very old individuals (Sokoloff et al., 2007, 2010; Daugherty et al., 2012).

Muscle mass reflects the balance between anabolic and catabolic processes, and perturbation of either with age may lead to sarcopenia (Arthur and Cooley, 2012). Studies primarily report stability of atrophy-related signaling and changes in growth-related signaling in muscle with age (i.e., Gaugler et al. 2011; Foletta et al., 2011) but also indicate variability by muscle, fiber type, species and gender (e.g., Foletta et al., 2011; Parkington et al., 2004). Our previous study in the rat demonstrated that, compared to the biceps brachii, pERK and p70S6k T421/S424 was preserved with age in head and neck muscles including the tongue muscle styloglossus. Whether growth-related signaling is maintained with age in human tongue muscles is not known. Rapid post-mortem degradation of protein phosphorylation status (e.g., Li et al., 2003) and comorbidities of human aging which affect muscle mass (e.g., chronic obstructive pulmonary disease, cancer, Ciciliot et al., 2013) complicate study of signaling pathways with age in human tongue muscles. Therefore we tested for anatomical and molecular indices of sarcopenia in the tongue muscle styloglossus of the macaque, a primate with relatively long life-span and tongue MHC composition similar to humans (Sokoloff et al., 2007). Our findings indicate minimal changes in measures typically associated with sarcopenia and suggest that primate styloglossus is resistant to common features of muscle aging.

2. Materials and methods

Whole tongues including extrinsic tongue muscles were removed immediately post-mortem from 12 male and 12 female macaques (*Macaca mulatta*) ranging in age from 0.9 months to 31 years (Table 1), frozen in liquid nitrogen and stored at −80 °C. Tissue was briefly thawed and the left or right styloglossus was removed immediately proximal to its entry into the tongue body to enable comparable sampling across subjects. SG tissue was mounted on tongue depressors with Tissue-Tek O.C.T. Compound (Sakura, Finetek), frozen in isopentane supercooled with liquid nitrogen and stored at −80 °C. Tissue was provided by the California National Regional Primate Center or the Yerkes Regional Primate Research. The study involved only post-mortem tissue and is Institutional Animal Care and Use Committee-exempt. Human tissue was obtained from a single, 80-year old cadaver through the Emory University School of Medicine Body Donor Program and is IRB-exempt.

2.1. Tissue preparation and SDS-PAGE-Coomassie of myosin heavy chain

Approximately 40–50 mg of muscle tissue was cut from frozen SG tissue blocks, homogenized in 200 μl of 0.1 M potassium phosphate (PBS) buffer (pH 7.3) and 5% protease inhibitor cocktail (Sigma, Aldrich) following Kohn and Myburgh (2006) with a tissue homogenizer (Fisher Scientific, PowerGen 500) in an ice bath, followed by centrifugation at 10,000 *g* (4 °C) for 10 min and re-suspended in 0.1 M PBS buffer (pH 7.3) and 5% protease inhibitor cocktail for extraction of the myosin fraction. Total protein content was assayed by bicinchoninic acid assay according to manufacturer specifications (Synergy HT multimode microplate reader, Biotek Instruments, Inc., Pierce® BCA protein assay,

Table 1
Subject information and experimental tests.

Subject information				Molecular tests			
Su Subject ID	Age months	Age category	Sex	Growth and atrophy signaling	MHC dev[a]	MHC Coomassie	MHC phenotype, morphometry
M1	9	Y	Male	+			
M4	47	Y	Male	+	+	+	
M6	47	Y	Male	+	+	+	+
M21	47	Y	Female	+	+	+	
M15	68	Y	Male	+	+	+	+
M16	72	Y	Male	+	+	+	+
M10	80	Y	Female	+	+	+	+
M26	89	Y	Male	+	+	+	+
M9	181	M	Male	+		+	+
M19	201	M	Female	+		+	
M11	204	M	Female	+		+	
M17	225	M	Female	+		+	+
M13	240	M	Male	+		+	
M33	249	M	Female	+		+	
M32	265	O	Male	+		+	+
M7	271	O	Female	+		+	+
M29	276	O	Male	+	+	+	+
M31	276	O	Male	+	+	+	+
M22	279	O	Female	+	+	+	+
M25	284	O	Male	+	+	+	+
M24	285	O	Female	+	+	+	+
M30	288	O	Female	+	+	+	+
M8	295	O	Female	+	+	+	+
M23	372	O	Female	+	+	+	+

[a] Developmental myosin heavy chain (MHC), MHCembryonic and MHCneonatal.