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Tongue exercise and ageing effects on morphological and biochemical properties of the posterior digastric and temporalis muscles in a Fischer 344 Brown Norway rat model

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ABSTRACT

Objective: This study sought to examin effects of age and tongue exercise on the posterior digastric (opener) and the temporalis (closer). We hypothesized 1) age would result in differing morphological (cross sectional area) and biochemical (myosin heavy chain isoform) components of these muscles; 2) tongue exercise would result in coactivation of these muscles inducing a decrease in age-related differences between age groups.
Design: Young adult (9 months) and old (32 months) Fischer 344 Brown Norway rats were randomized into a tongue exercise or control group. Post-training, posterior digastric and temporalis muscles were harvested and analyzed using: 1) Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to assess percent myosin heavy chain (MyHC) content; 2) Immunohistochemical staining to determine cross sectional area (CSA).
Results: A larger proportion of slowly contracting MyHC isoforms in the posterior digastric and temporalis muscles were found in old. No significant main effects for age or exercise in fiber size were found in posterior digastric muscle. An interaction between age and exercise for temporalis cross sectional area indicated the old exercise group had smaller average cross sectional area than all other groups.
Conclusions findings: suggest that: 1) Increasing age induces biochemical changes in muscles of the jaw, specifically showing an increase the proportion of slower contracting MyHC isoforms; 2) Increasing age and tongue exercise induce a reduction in muscle fiber cross sectional area in the temporalis muscle only. However, continued study of these cranial muscle systems is warranted to better understand these changes that occur with age and exercise.

1. Introduction

Aging results in progressive decrements in muscle function and can have a negative impact on deglutition (Newton, Abel, Robertson, & Yemm, 1987; Nicosia et al., 2000; Peyron, Blanc, Lund, & Woda, 2004; Roy, Stemple, Merrill, & Thomas, 2007; Schindler & Kelly, 2002). Age-related dysphagia is associated with reductions in quality of life and potentially serious complications such as aspiration pneumonia (Eisenstadt, 2010; Roy et al., 2007; Thein et al., 2009). Swallowing requires the coordination of many muscle groups, including those of the jaw and the tongue, which can be affected by sarcopenia (Rosenberg, 1997) and associated reductions in muscle strength, (Porter, Vandervoort, & Lexell, 1995; Saitoh et al., 2007) muscle size, (Rosenberg, 1997) fiber number, (Faulkner, Larkin, Claflin, & Brooks, 2007) and an increase in muscle fatigue (Thein et al., 2009). Within the human tonuge, intrinsic muscles critical to bolus propulsion have been shown to decrease in size (Nakayama, 1991). Animal studies have further demonstrated that changes in lingual muscle contractile and fiber properties (Schaser, Wang, Volz, & Connor, 2011) result in slower fiber contraction (Connor, Ota, Nagai, Russell, & Leverson, 2008). Muscles of mastication also show reductions in relative cross-sectional area with age (Galo, Vitti, Santos, Hallak, & Regalo, 2006; Newton, Yemm, & Menhinick, 1993).

Mastication involves a series of complex movements in coordination with the tongue that allows for bolus formation, saliva mixing, and ultimately, initiation of the swallow (Palmer, Rudin, Lara, & Crompton, 1992; Prinz & Lucas, 1997; Saitoh et al., 2007; Schindler & Kelly, 2002; Woda, Mishellany, & Peyron, 2006). Specifically, muscles of mastication, including the masseter, temporalis, pterygoid, and digastric (McLoon & Andrade, 2012) must act in concert with the intrinsic and extrinsic tongue muscles for successful bolus formation and swallowing (Schindler & Kelly, 2002).

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Older individuals often swallow more slowly, and for some there may be a reduction in tongue (Machida et al., 2017) and palatal pressure reserves that result in inadequate bolus formation and propulsion, (Steele & Cichero, 2014) increasing risk of aspiration (Robbins, Levine, Wood, Roecker, & Luschei, 1995). Physiological changes in mastication with aging include an increased number of masticatory cycles, (Mioche, Bourdiol, Monier, Martin, & Cormier, 2004; Peyron et al., 2004) decreased accuracy of bites, (Ballard, Robin, Woodworth, & Zimba, 2001) reduced bite force, (Bakke, Holm, Jensen, Michler, & Møller, 1990) and decreased jaw opening force (Iida et al., 2013). These changes may produce less efficient particalization of food, resulting in boli that are less comminuted (Mioche et al., 2004). The muscles of the tongue and the jaw work together simultaneously to effectively prepare and propel a bolus (Hiiemae, Hayenga, & Reese, 1995; Hori, Ono, & Nokubi, 2006; Kakizaki, Uchida, Yamamura, & Yamada, 2002; Naganuma, Inoue, Yamamura, Hanada, & Yamada, 2001). The structure and function of the tongue and jaw muscles are mutually impacted by age related decline (Galo et al., 2006; Newton et al., 1993; Newton et al., 1987; Porter et al., 1995).

Treatments for presbyphagia include tongue exercise with the goal of strengthening muscles of the tongue and improving palatal pressure generation and bolus propulsion during swallowing (Robbins et al., 1995). This treatment has been modeled in our laboratory in the rat, which allows study of biochemical and physiological changes that are not possible in humans (Connor et al., 2009; German, Crompton, Gould, & Thexton, 2017; Kletzien, Russell, Leverson, & Connor, 2013; Krekeler & Connor, 2016). We have demonstrated that tongue exercise increases generative force capacity in the tongue, and induces changes in intrinsic tongue muscle fiber type (Connor et al., 2009; Kletzien et al., 2013; Krekeler & Connor, 2016). Although muscles of the jaw have been shown to co-activate with muscles of the tongue, (Kayalioglu, Shcherbatyy, Seifi, & Liu, 2007; Yamamoto, Matsuo, Fujiwara, & Kawamura, 1982) our previous work demonstrated that tongue exercise did not have a significant impact on masticatory patterns in rats (Krekeler & Connor, 2016). However, It is not known if tongue exercise induces biochemical changes in the muscles of the jaw that were not detected using behavioral measures of mastication. The hypothesis that increased tongue strength could impact masticatory function has been supported in the literature. Specifically the tongue and jaw coordinate during masticatory functions in bolus preparation and transit (Hiiemae et al., 1995; Hori et al., 2006; Kakizaki et al., 2002; Naganuma et al., 2001). Thus, increased tongue strength could provide better lingual support for masticatory functions and improve timing sequelae in both chewing and swallowing.

This gap in knowledge is important to explore because tongue exercise in our model approximates tongue exercise treatments used to treat dysphagia in humans. Therefore, to gain a full understanding of the potential biological effects of this treatment, we must study exercise effects on the cellular level (German et al., 2017). Because feeding and mastication are both critical components of the oropharyngeal swallow, it is necessary to understand the implications of tongue exercise on supporting systems in the mascitatory musculature.

In a previous study (see: Krekeler & Connor, 2016), we examined behavioral effects of age and tongue exercise on masticatory function. The purpose of this follow up study is to examine the effects of age and tongue exercise on biochemical and morphologic properties of two muscles of mastication, the posterior digastric and the temporalis. The digastric muscle is involved in opening of the jaw and the temporalis is involved in jaw closure; together these muscles work along with others to form a complete masticatory cycle. We hypothesized that: (1) muscle morphology (cross sectional area) and biochemistry (myosin heavy chain isoform proportions) would differ in young adult vs old rat groups; specifically, that age would be associated with a decrease in cross sectional area and a shift from fast-contracting isoforms to more slowly contracting isoforms; and, (2) tongue exercise would induce morphometric and biochemical changes in the posterior digastric muscle and the temporalis muscle; specifically, that tongue exercise would be associated with a decrease in age-related differences between the young adult and old rat groups (De-Ary-Pires, Ary-Pires, & Pires-Neto, 2003; Hiiemae & Houston, 1971).

2. Methods

2.1. Animal exercise and mastication measure

The University of Wisconsin School of Medicine and Public Health Animal Care and Use Committee approved all procedures. Animal subjects were 34 male Fischer 344 Brown Norway rats (17 young adult rats [9-months] and 17 old rats [32-months]). Animal training and exercise procedures are reported in detail elsewhere (Krekeler & Connor, 2016). Briefly, rats were randomized into control and exercise groups with 9 tongue exercise animals and 8 control animals per age group. Mastication testing was performed at two time points: before exercise training was initiated and at the end of the 8-week training period. Mastication testing was performed using a pasta-biting task previously described (Allred et al., 2008; Kane et al., 2011; Plowman et al., 2013; Tennant et al., 2010). The pasta biting task involved quantifying number of bites, time to eat, and interbite interval using acoustic signals emitted from the rats biting uncooked pasta pieces. After pre-training data were collected, rats underwent the tongue exercise program over 8 weeks that involved progressively increasing the tongue force required to receive a water reward. Rats underwent a water-restriction protocol to provide motivation for pressing the tongue on the disc to receive a water reward. Tongue force targets were progressively increased force targets over 8 weeks. This progressive increase of force targets in an 8 week training period mirrors the human tongue training intervention (Robbins et al., 2005). Post-training pasta biting data were collected at completion of 8 weeks of tongue exercise.

2.1.1. Tissue preparation

Following post-training pasta biting testing, rats were euthanized following anesthesia with isoflurane using Beuthanasia via intraperitoneal injection (0.2 cc). The paired posterior digastric and paired temporalis muscles were harvested. Each of the paired sides of the temporalis muscle were randomized to the myosin heavy chain (MyHC) assay or the muscle fiber cross sectional area (CSA) assay. The two paired bellies of the posterior digastric lie very closely together and therefore are difficult to separate without tearing. Thus the digastric muscle bellies were analyzed together, and were prepared first for CSA analysis, then homogenized for MyHC analysis. Tissues to be used for CSA analysis were frozen in an optimal cutting temperature (OCT) compound, while tissues bound for MyHC analysis were snap frozen in liquid nitrogen. All prepared tissues were kept frozen at −80 °C until analyzed. All muscles were processed using standard lab protocols for CSA and MyHC analysis using the extensor digitorum longus (EDL) and soleus (SO) muscles as control tissues (Connor et al., 2009).

2.1.2. Myosin heavy chain analysis with SDS-PAGE

Myosin heavy chain (MyHC) analysis was performed using a Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) assay to determine the distribution of MyHC isoforms in the muscle. Because MyHC composition is highly linked to muscle fiber type, (Scott, Stevens, & Binder–Macleod, 2001) this analysis allowed us to infer the types of muscle fibers present and their associated contraction speeds and force generation capacities (Pette & Staron, 2000). MyHC isoforms studied in muscles of the rat include MyHC type I, IIb, IIx, and IIa and separate in this order in a gel (Larsson & Ansved, 1995). First, muscle tissue fibers were homogenized, muscle proteins were extracted and analyzed for protein concentration (Bradford Protein Assay) using 0.4 μg of protein per well. Then, protein aliquots of 4 μL (0.4 μg total concentration) were processed using SDS-PAGE (0.75-mm-thick 6% acrylamide-30% glycerol separating gel, 18 × 16 cm, and a 4% acrylamide-30% glycerol