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Sexual dimorphism of murine masticatory muscle function

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ABSTRACT

Objective: To determine if gender distinctions of force generating capacity existed in murine masticatory muscles.
Design: In order to investigate the effect of sex on force generating capacity in this muscle group, an isolated muscle preparation was developed utilising the murine anterior deep masseter. Age-matched male and female mice were utilized to assess function, muscle fibre type and size in this muscle.
Results: Maximum isometric force production was not different between age-matched male and female mice. However, the rate of force generation and relaxation was slower in female masseter muscles. Assessment of fibre type distribution by immunohistochemistry revealed a three-fold decrease in the proportion of myosin heavy chain 2b positive fibres in female masseters, which correlated with the differences in contraction kinetics.
Conclusions: These results provide evidence that masticatory muscle strength in mice is not affected by sex, but there are significant distinctions in kinetics associated with force production between males and females.

1. Introduction

Craniofacial muscles are distinct from those in the limbs and trunk in both their developmental origin and their protein/molecular composition. Muscles of the craniofacial region arise from the head prechordal mesoderm or neural crest, whereas limb and trunk muscle originate in the paraxial mesoderm flanking the neural tube.[1] In addition, human masticatory muscles present a unique fibre type and size distribution compared to limb and trunk muscles,[2–4] a feature which has also been documented in many other species, including mice, rabbits, and pigs.[5–7]

The process of mastication involves several different muscles to open and close the jaw as well as laterally position the teeth. In humans, the masseter, temporalis and medial pterygoid muscles are the primary jaw closers, which develop the most force and are the site of pain after chewing. In rodents, it is the masseter that is the major force-producing jaw-closing muscle, which has been demonstrated by electromyographic monitoring of all muscles of mastication in rats.[8] The masseter muscle fibre orientation is not uniform and can be parallel or perpendicular to the temporomandibular joint (TMJ). Therefore, *in situ* examination of function must account for multiple fibre direction and the types of movements generated by contraction, or must examine the force of jaw closure as an indirect method of muscle contractile force.

Several *in vivo* models have been utilized to assess functional changes in mastication and have provided insight

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Abbreviations: ADM, anterior deep masseter; DAPI, 4′-6-diamidino-2-phenylindole; dP/dt, rate of force generation; FSHD, facioscapulohumeral muscular dystrophy; Lo, optimum muscle length; MHC, myosin heavy chain; Po, maximum isometric tetanic force; Pt, maximum isometric twitch force; SP force, force per cross-sectional area; TMD, temporomandibular disorders; TMJ, temporomandibular joint; 1/2 Rel, relaxation time to 50% maximum force after stimulation.

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into the myogenic component of muscle disorders such as those involving the TMJ.[9,10] An *ex vivo* model enables study of contractile characteristics of the muscle independent of changes in neural input or tissue environment, and may aid in determining the role of the contractile apparatus in the onset of TMD.

The goal of this investigation was to determine if the murine masticatory muscle function differs by sex. To date, this has been examined at the single fibre level, but not in an isolated whole muscle. Therefore, we developed an isolated anterior deep masseter (ADM) preparation to determine if force generation differs between male and female mice.

2. Materials and methods

All procedures were approved by the University of Pennsylvania IACUC. Adult C57Bl/6 mice ($N = 8$ per sex, age 6 months), a widely accepted strain for muscle testing, were utilized for this study. Mice were sacrificed by carbon dioxide asphyxiation. The superficial masseter tendon was cut from the mandible, and the muscle was pulled back and removed to reveal the deep masseteric region. The mandible and the zygomatic process of the maxilla were cut at the sites shown in Fig. 1, maintaining the deep masseter between these bones. The entire bone–muscle unit was separated from the rest of the skull and placed in oxygenated (95% O_2 and 5% CO_2) Ringer's solution, maintained at pH 7.4 and 22 °C. Each sample was trimmed under a dissecting microscope along the direction of the fibres to a width of approximately 2 mm, revealing an origin at the rostral maxillary portion of the zygomatic arch and an insertion along the masseteric ridge of the mandible. Silk sutures were threaded over the four bony corners of the sample, forming superior and inferior ''trapezes'' for suspension. Fig. 1 illustrates the site of the dissected muscle and the preparation of the ADM for mechanical measurements.

The ADM muscle preparation was attached by the sutures to a fixed hook and a force transducer (Model 300C; Aurora Scientific, Ontario, CAN) within a temperature controlled bath of oxygenated Ringers, pH 7.4, 22 °C as previously described.[11] The suspended muscle was held between two platinum electrodes (Aurora Scientific, Ontario, CAN). Supramaximal, single pulse stimulations determined the optimal muscle length (Lo). Muscle length was determined between the bony insertions at the centre of the muscle preparation using callipers. The ADM then received a single pulse followed by a train of 120 Hz–800 ms pulses 5 s later to measure maximum isometric twitch (Pt) and tetanic (Po) force, respectively. Initial experiments determined that this frequency was sufficient to generation fused tetanic stimulation in both male and female muscle preparations (data not shown). The stimulation pattern was delivered three times with 5 min rest between each measurement. The muscles were then placed in a fresh bath of oxygenated Ringer's solution containing 0.1% procion orange (Reactive Orange 14; Sigma–Aldrich, St Louis, MO) for 20 min to enable visualisation of fibres at each edge of the muscle preparation that had been damaged during the dissection.[12] This is a membrane impermeant dye that can only enter fibres when the sarcolemma is disrupted. Muscle samples were washed in Ringers without procion orange, then dissected free of the bones. All tissue samples were blotted, weighed, pinned at resting length and the orientation of the fibres were aligned along the length of the mounting block. Mounting samples were embedded in mounting medium (Tissuetek; Sakura, Torrance, CA), and then rapidly frozen in melting isopentane and stored at −80 °C for morphological analysis.

Absolute cross-sectional area was determined using the muscle mass, Lo, and the density of muscle (1.06 g/mm^3) (mass/(Lo × muscle density)), and used to calculate maximum specific force (force/cross-sectional area, SP force). The calculated cross-sectional area was confirmed with image analysis (see below). Force generation analysis was performed using software associated with the mechanics apparatus (DMA Version 3.2; Aurora Scientific, Aurora, Ontario, CAN).

Frozen muscle cross-sections (10 μm) were utilized to assess damaged fibres, distribution of fibre area, and fibre type. Orientation of the cross-section was confirmed during sectioning under a light microscope. Image acquisition and analysis were performed on an epifluorescence microscope (Leica DMR, Bannockburn, IL) and associated software (OpenLab; Improvision, UK). For each muscle, an image of the entire cross-section was acquired. Slides utilized for analysis of fibre damage were washed in phosphate-buffered saline (PBS), air-dried, then covered with mounting media containing 4′-6-diamidino-2-phenylindole (DAPI, Vectashield, Burlingame, CA) to identify nuclei. Damaged fibres were ones that had allowed procion orange entry. The proportion of the muscle at the mid-belly that had damaged fibres was calculated and subtracted from the absolute cross-sectional area to determine the functional cross-sectional area. Muscle preparations with more than 15% damage were eliminated from the study. Fibre area analysis was achieved using immunohistochemistry and an antibody recognising laminin as previously described.[13] The imaging software provided further confirmation that the fibres were not

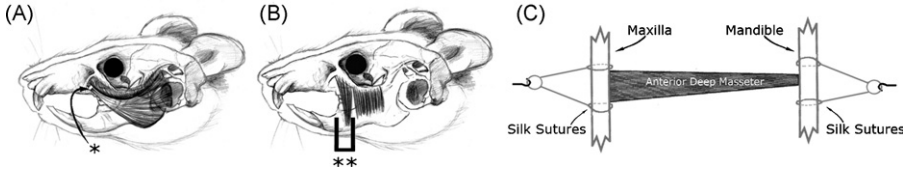

Fig. 1 – Process of preparation of ADM for isolated muscle mechanics. (A) Removal of superficial masseter occurs by cutting the tendon (*) to reveal the deep regions of the masseter. (B) Region of the deep masseter (**) utilized for isolated muscle mechanical measurements. (C) Suture attachment sites on the mandible and zygomatic process of the maxilla flank the region of muscle to be utilized for functional measurements.