Specific Force of the Rat Extraocular Muscles, Levator and Superior Rectus, Measured In Situ

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Frueh, Bartley R., Paul Gregorevic, David A. Williams, and Gordon S. Lynch. Specific force of the rat extraocular muscles, levator and superior rectus, measured in situ. J Neurophysiol 85: 1027–1032, 2001. Extraocular muscles are characterized by their faster rates of contraction and their higher resistance to fatigue relative to limb skeletal muscles. Another often reported characteristic of extraocular muscles is that they generate lower specific forces ($sP_o$, force per muscle cross-sectional area, kN/m$^2$) than limb skeletal muscles. To investigate this perplexing issue, the isometric contractile properties of the levator palpebrae superioris (levator) and superior rectus muscles of the rat were examined in situ with nerve and blood supply intact. The extraocular muscles were attached to a force transducer, and the cranial nerves exposed for direct stimulation. After determination of optimal muscle length ($L_o$, expressed as a percentage of overall muscle length, allowing a mean $L_o$ to $L_f$ ratio to be used in the estimation of muscle cross-sectional area. Mean $L_o$/$L_f$ was determined to be 0.38 for the levator muscle and 0.45 for the superior rectus muscle. The $sP_o$ for the rat levator and superior rectus muscles measured in situ was 275 and 280 kN/m$^2$, respectively. These values are within the range of $sP_o$ values reported for rat skeletal muscles. Furthermore, $P_o$ and $sP_o$ for the rat levator and superior rectus muscles measured in situ were significantly higher ($P < 0.001$) than those of the cat inferior oblique muscles from the cat measured in situ. Hanson and Lennerstrand (1977) investigated the contractile properties of the inferior oblique muscle of the cat measured in situ. Although $P_o$ was not stated, it can be estimated to be 1.4 g ($\sim$13.7 mN) in the rat and 11.3 g ($\sim$111 mN) in the cat. This $P_o$ for the cat inferior oblique is much lower than that reported by Bach-y-Rita and Ito (1966).

The issue of whether extraocular muscles produce lower specific force than skeletal muscles has been addressed partly in the elegant studies by Goldberg and colleagues (Goldberg et al. 1994). One of the most perplexing issues in muscle physiology is that extraocular muscles generate significantly lower specific forces ($sP_o$, force per muscle cross-sectional area, kN/m$^2$) than skeletal muscles. Previous studies on extraocular muscle contractility have used muscle length ($L_o$) in the calculation of muscle cross-sectional area, a procedure that actually leads to an overestimation of $sP_o$. These studies have reported $sP_o$ values in the range of 7–110 kN/m$^2$ (Asmussen and Gaunitt 1981; Asmussen et al. 1994; Close and Luff 1974; Frueh 1985; Frueh et al. 1994; Luff 1981), significantly less than the 220–300 kN/m$^2$ reported for limb skeletal muscles of rats (Devor and Faulkner 1999) and mice (Lynch et al. 1999) that have used muscle fiber length ($L_f$) in the calculation of muscle cross-sectional area. In these previous studies, the examination of extraocular muscle contractility was generally performed on isolated muscles in vitro. It is possible that during the intricate dissections required for in vitro investigation that surgical trauma results in damage directly to the extraocular muscles and this contributes to the disparity in $sP_o$ values between extraocular and skeletal muscles (Frueh 1985). To specifically address the issue of whether the intrinsic force development is different between extraocular and other skeletal muscles, assessment of muscle function can be performed in situ, i.e., where the muscles contract following direct stimulation of the nerve and where blood supply to the muscle is not compromised. Although there is generally good agreement among investigators who have studied extraocular muscle forces in situ (Bach-y-Rita and Ito 1966; Barmack et al. 1971; Cooper and Eccles 1930), none have reported $sP_o$ of the inferior oblique muscles from cats, in situ to be 40 g ($\sim$390 mN). Barmack et al. (1971) reported a peak fusion tension of 110 g ($\sim$1000 mN) for the lateral rectus muscle of the cat measured in situ. Although $P_o$ was not stated, it can be estimated to be 1.4 g ($\sim$13.7 mN) in the rat and 11.3 g ($\sim$111 mN) in the cat. This $P_o$ for the cat inferior oblique is much lower than that reported by Bach-y-Rita and Ito (1966).

INTRODUCTION

The contractile properties of the extraocular muscles differ significantly from typical skeletal muscles. Numerous studies have shown that despite being among the muscles with the fastest speed of contraction (Porter and Baker 1996) the extraocular muscles are relatively resistant to fatigue (Frueh et al. 1994). One of the most perplexing issues in muscle physiology is why extraocular muscles generate significantly lower specific forces ($sP_o$, force per muscle cross-sectional area, kN/m$^2$) than skeletal muscles. Previous studies on extraocular muscle contractility have used muscle length ($L_o$) in the calculation of muscle cross-sectional area, a procedure that actually leads to an overestimation of $sP_o$. These studies have reported $sP_o$ values in the range of 7–110 kN/m$^2$ (Asmussen and Gaunitt 1981; Asmussen et al. 1994; Close and Luff 1974; Frueh 1985; Frueh et al. 1994; Luff 1981), significantly less than the 220–300 kN/m$^2$ reported for limb skeletal muscles of rats (Devor and Faulkner 1999) and mice (Lynch et al. 1999) that have used muscle fiber length ($L_f$) in the calculation of muscle cross-sectional area. In these previous studies, the examination of extraocular muscle contractility was generally performed on isolated muscles in vitro. It is possible that during the intricate dissections required for in vitro investigation that surgical trauma results in damage directly to the extraocular muscles and this contributes to the disparity in $sP_o$ values between extraocular and skeletal muscles (Frueh 1985). To specifically address the issue of whether the intrinsic force development is different between extraocular and other skeletal muscles, assessment of muscle function can be performed in situ, i.e., where the muscles contract following direct stimulation of the nerve and where blood supply to the muscle is not compromised. Although there is generally good agreement among investigators who have studied extraocular muscle forces in situ (Bach-y-Rita and Ito 1966; Barmack et al. 1971; Cooper and Eccles 1930), none have reported $sP_o$ of the inferior oblique muscles from cats, in situ to be 40 g ($\sim$390 mN). Barmack et al. (1971) reported a peak fusion tension of 110 g ($\sim$1000 mN) for the lateral rectus muscle of the cat measured in situ. Although $P_o$ was not stated, it can be estimated to be 1.4 g ($\sim$13.7 mN) in the rat and 11.3 g ($\sim$111 mN) in the cat. This $P_o$ for the cat inferior oblique is much lower than that reported by Bach-y-Rita and Ito (1966).
and Shall 1997; Goldberg et al. 1997; Gurahian and Goldberg 1987; Meredith and Goldberg 1986; Shall and Goldberg 1992; Shall et al. 1995), which reported in situ force output of individual motor units in the lateral rectus, inferior oblique, and medial rectus muscles of the cat. There is also generally good agreement among investigators (including Goldberg and colleagues) regarding motor unit forces in cat extraocular muscles (Nelson et al. 1986; Waldeck et al. 1995). Based on the delineation of average twitch tension related to motor unit type, Shall and Goldberg (1992) found that only one-half of the twitch response was evident during actual stimulation of whole nerve. They hypothesized that the serial arrangement and branching of cat lateral rectus muscle fibers (Alvarado-Mallart and Pincon-Raymond 1976) led to lower than expected muscle unit forces when adjacent muscle units contracted (Goldberg et al. 1997). In another study, Goldberg and Shall (1997) measured contractile properties of the intact lateral rectus muscle from two cats in situ, via stimulation of the sixth cranial nerve, and again found that $P_o$ was significantly less than would be predicted by linear summation of individual motor unit twitch and maximal tetanic forces. Unfortunately, $L_o$, $L_T$ or muscle mass were not reported that would have enabled an estimation of $sP_o$ (Goldberg and Shall 1997).

In this study, we have re-examined the issue of whether extraocular muscles produce lower specific forces than skeletal muscles. Specifically we have investigated the force producing capacity of the extraocular muscles, the levator and superior rectus, from the rat, in situ. We then compared the values for $P_o$ and $sP_o$ with those for muscles studied in vitro. We tested the null hypothesis that the specific force for extraocular muscles obtained in situ would not be different from that of limb skeletal muscles. A corollary to our primary hypothesis was that absolute and specific forces for extraocular muscles obtained in situ and in vitro would not be different.

**METHODS**

All experimental procedures performed were approved by the Animal Experimentation Ethics Committee of The University of Melbourne. Male and female Sprague-Dawley rats (250–450 g) were used in these studies.

For the evaluation of extraocular muscle function in situ, the rats were anesthetized deeply with intraperitoneal injections of sodium pentobarbitone of (Nembutal, Rhone Merieux, Pinkenba, QLD, Australia, 60–80 mg/kg) such that they did not respond to tactile stimuli throughout the procedures. This depth of anesthesia was monitored carefully and maintained with supplemental injections of sodium pentobarbitone. The skin and subcutaneous tissue was excised over the calvarium. The temporals muscle and fascia was separated from the skull and subtotally excised. The calvarium was reduced to tissue-paper thinness with a power-driven burr. The thin remaining skull bone was separated from the dura and excised from anterior to the posterior suture lines to the beginning of the ophthalmic bulbs. Laterally bone was removed to the region at which it thickens markedly below the point of insertion of the temporalis muscle, exposing much of the anterior brain. If the dura had not been opened in the course of bone removal, it was opened at this time. The sagittal sinus and visible surface vessels were cauterized. The anterior brain was lightly retracted from each side, and the middle meningeal artery was cauterized. The brain was vertically transected with a hot cautery in front of the posterior skull suture lines, and the anterior brain similarly was separated from the ophthalmic bulbs. The optic nerves were cut and the anterior brain excised. Bleeding on the cut surface of the brain was cauterized. This procedure exposed cranial nerves III–VI as they ran along the floor of the brain cavity just prior to entering the orbit. Cranial nerve attachments to the brain stem were not disrupted. The anesthetized rats maintained cardiac and respiratory function throughout the evaluation procedures following removal of their anterior brain.

The levator palpebrae superioris (levator) muscle was isolated from a randomly chosen side. The upper eyelid was transected medial and lateral to the fornix. A 4–0 silk suture was placed through the anterior portion of the tarsal plate centrally. This suture was later used to fix the tarsus and hence the tendon of the levator muscle to the force transducer. The lid was retracted carefully with the suture. Antero-posterior conjunctival/Tenons capsule incisions were made on either side of the superior rectus muscle, and a conjunctival peritomy was performed distal to the insertion of the superior rectus muscle. The superior oblique muscle was identified and cut. The insertion of the superior rectus muscle was looped with a 4–0 silk suture and pulled down carefully, rotating the eye forward. Conjunctiva was incised across the fornix, and all attachments between the superior rectus and the levator muscles were lysed sharply. The insertional tendon of the superior rectus muscle was isolated, tied, and cut from the globe. The superior rectus muscle was retracted, and the globe was pulled forward to expose the retractor bulbis muscles and optic nerve, which were then cut from the globe. The tendons of insertion for the lateral rectus, medial rectus, inferior rectus, and the inferior oblique muscles were all severed and the eye was removed. The anterior two-thirds of the superior rectus muscle was amputated, and the remainder was allowed to retract.

On the contralateral side, the superior rectus muscle was isolated from the other extraocular muscles. Dissection was similar to that performed to isolate the levator muscle except that the superior rectus muscle was not amputated. However, the suture looping the tendon of the superior rectus muscle was tightly tied around it prior to cutting the tendon from the globe, and this suture was later used to attach the tendon to the force transducer. Additionally, the upper eyelid and the anterior two-thirds of thelevator muscle were excised, and the remainder of the levator muscle was allowed to retract.

After the muscle dissections were completed, the anesthetized, decorticatate rat was mounted on a stereotaxic unit with the head rigidly fixed by the front incisors, a clamp on the nose, and blunt pins contacting the interior of the ear canals. The suture attached to the tendon of the muscle to be tested was looped through the arm of a calibrated isometric force transducer (Research Grade 60-2999, Harvard Apparatus, South Natick, MA). The transducer arm was adjusted (via $x$, $y$, and $z$-axis micromanipulation) to ensure alignment with the muscle. The plane of tension measurement was $-45^\circ$ to the midline of the animal. Preliminary testing revealed that the highest force was obtained with the transducer aligned $-45^\circ$ up from the horizontal plane of the animal’s head. The muscle was then tied tightly to the transducer arm, and the arm was retracted with a vernier-scaled micromanipulator until the muscle was adjusted to a just-taut length. Two flexible platinum wires with bulbous ends, separated by 2 mm, were connected to a square wave stimulator (S48 Grass Instruments, Quincy, MA). The electrodes were positioned within the anterior cranium using an X-Y micromanipulator, such that both ends contacted the medial side of the bundle of cranial nerves III–VI of the side being tested, $-5$ mm proximal to the point of entry to the orbit. The nerves were stimulated with $6–8$ V pulses (0.2-ms duration). Contractile measurements were recorded with a four-channel PowerLab recorder (ADInstruments, Castle Hill, N.S.W., Australia) run by a personal computer (PII, Paragon Computers, Australia) operating Chart data acquisition software (v0.3.4.6, ADInstruments, Castle Hill, N.S.W., Australia).

Optimal muscle length ($L_o$) and stimulation voltage were determined from micromanipulation of muscle length and a series of twitch contractions that produced maximum isometric twitch force ($P_o$). A full frequency-force relationship was determined by stimulating the
muscles at frequencies of 1, 5, 10, 20, 30, 50, 80, 100, 120, 150, 180, 200, 250, 300, and 350 Hz. Muscles were rested for 2.5 min between tetanic stimuli. Maximal $P_{o}$ was determined from the plateau of the frequency force relationship. The muscles were then stimulated once every 5 s for a 4-min period, using the optimal parameters for voltage and frequency determined previously. The position of the insertion and the origin of the muscle were estimated, and optimum muscle length ($L_o$) was determined as the distance between those two points.

Following completion of muscle testing, the attachment suture was then severed, and the transducer was moved to the contralateral side of the animal to test the other muscle in an identical fashion. At the conclusion of the contractile measurements, each muscle was dissected carefully, trimmed of nonmuscle tissue, blotted on filter paper, and then weighed on an analytical balance. The animals were killed by cervical dislocation while still under deep anesthesia. In one experiment, after the frequency-force relationship of one superior rectus and onelevator muscle had been determined in situ, each muscle was dissected from its origin and transferred immediately into a custom-built Plexiglass bath filled with Krebs Ringer solution [which contained (in mM) 137 NaCl, 24 NaHCO$_3$, 11 d-glucose, 5 KCl, 2 CaCl$_2$, 1 NaH$_2$PO$_4$, 1 MgSO$_4$, and 0.025 d-tubocurarine chloride] that was bubbled with Carbogen (5% CO$_2$ in oxygen, BOC, Preston, Victoria, Australia) and thermostatically maintained at 25°C. The contractile properties of the muscles were then assessed in vitro, using techniques described previously (Lynch et al. 1999). The muscles were tied directly between a fixed immovable hook and the same force transducer used for the in situ evaluations. Muscles were field stimulated by supramaximal square-wave pulses (0.2 ms duration, S48 stimulator, Grass Instruments), that were amplified (EP500B Ebony power amplifier, Audio Assemblers Pty, Campbellfield, Victoria, Australia), and delivered to two platinum plate electrodes that flanked the length of the muscle to produce a maximum isometric tetanic contraction. After determination of $L_o$ and optimal voltage, maximum $P_o$ was determined from the plateaus of the frequency-force relationship following stimulation at increasing frequencies, with 2.5-min rest between stimuli.

Estimation of muscle cross-sectional area

The mean fiber lengths ($L_o$) in the levator and superior rectus muscles of the rat were determined using methods adapted from Segal et al. (1986). Onelevator and one superior rectus muscle was dissected from each of three rats. The six muscles were pinned at resting length in a dish with a base of 184 silicone elastomer (Sylgard, Dow Corning, Midland, MI) and filled with 4% paraformaldehyde in phosphate-buffered saline (PBS). After 3 days, the paraformaldehyde was aspirated, the muscle washed three times with PBS, and the muscle then covered with a 20% nitric acid solution. After 5 days, the nitric acid was aspirated and the muscle washed three times with PBS. The muscle (still pinned in the dish) was then covered with a solution of 50% glycerol with 0.1% sodium dodecyl sulfate. Muscle length was determined using calibrated digital calipers with an accuracy within 0.01 mm. As many fibers as possible were separated under a microscope using either a pair of jeweler’s forceps or fine-tipped glass micropipettes. The fiber lengths were recorded as a proportion of the final length of the muscle following storage in the 20% nitric acid solution. $L_f$ was determined from the fibers that could be sampled from the three superior rectus and three leveror muscles.

Muscle mass, $L_f$ and $P_o$, were used to calculate maximum specific isometric tetanic force ($sP_o$) or maximum $P_o$, normalized per total muscle cross sectional area (kN/m$^2$). The total fiber cross-sectional area (CSA) of each muscle was estimated by dividing muscle mass (mg) by the product of the determined mean $L_f$ and 1.06 mg/mm$^2$, the

| TABLE 1. Contractile properties of the levator and superior rectus muscles of the rat evaluated in situ |
|---|---|---|
| Levator | Superior Rectus |
| n | 5 | 5 |
| Muscle mass, mg | $1.9 \pm 0.2$ | $4.2 \pm 0.4$ |
| $L_o$, mm | $7.3 \pm 0.1$ | $9.3 \pm 0.2$ |
| $P_o$, mN | $47 \pm 7$ | $51 \pm 6$ |
| $sP_o$, kN/m$^2$ | $177 \pm 13$ | $266 \pm 27$ |
| $P_o/P_o$, | $0.27 \pm 0.04$ | $0.19 \pm 0.03$ |
| Fatigue, % | $31 \pm 13$ | $28 \pm 11$ |

Values are means $\pm$ SE. n, number of muscles; $L_o$, muscle length; $P_o$, twitch force; $P_o$, maximum tetanic force; $sP_o$, specific force (force per muscle cross-sectional area, see METHODS); fatigue, force output at the conclusion of the 4-min stimulation protocol, as a percentage of initial (pre-fatigue) force.
density of mammalian skeletal muscle (Méndez and Keys 1960). Absolute \(P_o\) values were normalized for muscle CSA using the formula \(sP_o (kN/m^2) = P_o (mN)/CSA (mm^2)\). Values are presented in the text and tables as means \(\pm SE\) unless stated otherwise.

**RESULTS**

**Calculation of fiber length to muscle length ratio**

For the superior rectus muscle, the ratio of \(L_f\) to \(L_o\) based on a sample of 269 fibers, ranged from 0.25 to 0.81, with a mean value of 0.45 \(\pm 0.16\) (SD). The distribution of \(L_f/L_o\) values for the superior rectus muscle is presented in Fig. 1A. For the levator muscle, the \(L_f/L_o\) based on a sample of 65 fibers, ranged from 0.17 to 0.85, with a mean value of 0.38 \(\pm 0.08\) (SD). The distribution of \(L_f/L_o\) values is presented in Fig. 1B.

**Contractile properties of extraocular muscles studied in situ**

The contractile properties of the levator and superior rectus muscles evaluated in situ are detailed in Table 1. Representative force traces for each of the two muscles are presented in Fig. 2. For comparative purposes, Table 2 lists some isometric contractile properties of one levator muscle and five isolated rat superior rectus muscles evaluated in vitro. The \(sP_o\) for the levator muscles measured in situ, 275 kN/m\(^2\), was significantly higher than that measured in vitro, 10 kN/m\(^2\) (\(P < 0.001, 2\)-tailed \(t\)-test). The mean \(sP_o\) for the superior rectus muscles measured in situ, 280 kN/m\(^2\), was significantly higher than that measured in vitro, 27 kN/m\(^2\) (\(P < 0.001, 2\)-tailed \(t\)-test).

In one experiment, the levator and superior rectus muscles of one rat were tested first in situ and then tested immediately in vitro. The \(sP_o\) for the levator muscle was 222 kN/m\(^2\) in situ and 7.5 kN/m\(^2\) in vitro. For the superior rectus muscle, the specific force was 270 kN/m\(^2\) in situ and 39 kN/m\(^2\) in vitro.

**DISCUSSION**

The most important finding of this study was that the specific force of extraocular muscles examined in situ was similar to the specific force of limb skeletal muscles. The extraocular muscles have always been considered to produce low forces, based primarily on data obtained from isolated muscles evaluated in vitro. The \(sP_o\) values for the levator and superior rectus muscles (275–280 kN/m\(^2\)) are within the range of \(sP_o\) values for in vitro and in situ recordings from skeletal muscles from a variety of different species including the mouse (Brooks et al. 1995; Lynch et al. 1999) and rat (Brown and Hasser 1996; Devor and Faulkner 1998; Gregorevic et al. 2000; Van der Meulen et al. 1997). Our original hypothesis that the specific force for extraocular muscles obtained in situ would not be different from that of limb skeletal muscles was supported. Our findings are important because they revise our understanding of the contractile properties of extraocular muscles.

Previous studies that have reported \(sP_o\) for extraocular muscles have conducted their experiments in vitro (Asmussen and Gaunitz 1981; Asmussen et al. 1994; Close and Luff 1974; Frueh et al. 1994; Luff 1981). Our data for force output of extraocular muscles in vitro (Table 2) are within the range previously reported. While the data for extraocular muscles evaluated in vitro can still provide important information about the contractility of these muscles, they clearly give a poor estimate of the true \(sP_o\). Because the \(sP_o\) values reported in this study are about six times greater than the highest of those reported previously for extraocular muscles in vitro even after the reduction caused by computing the \(sP_o\) of muscles using \(L_f\) rather than \(L_o\), our data require careful evaluation. This includes examination of potential sources of error, including the angle at which force is recorded in relation to the muscle’s position within the head, co-contraction of nearby muscles, and measurement of critical factors used in the calculation of \(sP_o\): absolute \(P_o\) and muscle fiber length.

**Consideration of the anatomical location of the extraocular muscles being tested**

Both the levator and superior rectus muscles rise in an arc over the eye in the natural state. We found that this angle of rise was critical to the measurement of maximum force. When the muscle force was measured with the muscle horizontal (parallel to the horizontal plane of the animal) and then at two levels (\(-22^\circ\) and \(-45^\circ\)) above the horizontal, \(P_o\) of the superior

**TABLE 2. Contractile properties of the superior rectus muscles of the rat evaluated in vitro**

<table>
<thead>
<tr>
<th></th>
<th>Superior Rectus</th>
<th>Levator</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Muscle mass, mg</td>
<td>2.7 (\pm) 0.6</td>
<td>4.4</td>
</tr>
<tr>
<td>(L_o), mm</td>
<td>8.0 (\pm) 0.6</td>
<td>7.5</td>
</tr>
<tr>
<td>(P_o), mN</td>
<td>20.4 (\pm) 3.9</td>
<td>15.0</td>
</tr>
<tr>
<td>(sP_o), kN/m(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncorrected</td>
<td>58.3 (\pm) 6.4</td>
<td>27.1</td>
</tr>
<tr>
<td>Corrected</td>
<td>27 (\pm) 2</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Values are means \(\pm SE\). \(n\), number of muscles; \(L_o\), muscle length; \(P_o\), maximum tetanic force; \(sP_o\), specific force (force per muscle cross-sectional area, see METHODS); uncorrected (\(sP_o\) not corrected for \(L_f/L_o\)); corrected (\(sP_o\) corrected for \(L_f/L_o\)).

**FIG. 2.** Typical traces of peak isometric twitch (\(P_t\)) and tetanic (\(P_P\)) tension produced by the superior rectus (\(A\)) and levator (\(B\)) muscles of the rat measured in situ following direct simulation of the cranial nerves (see METHODS). Maximum tetanic response was attained at a stimulation frequency of 200 Hz.
rectus muscle increased serially to 203% of the horizontal \( P_o \). With the levator muscle, the angle was increased twice, starting from a position just above the horizontal, and \( P_o \) increased serially to 187% of the initial \( P_o \). With the anesthetized animal mounted horizontally on the stereotaxic equipment, the optimal plane of tension development was \( \sim 45^\circ \) above the horizontal and \( \sim 45^\circ \) lateral to the midline of the animal. Clearly identifying the appropriate angle between the orbit and the extraocular muscle under consideration (to mimic that in the natural state) is critical for the determination of force output in situ. We found that any deviation from the optimal angle caused \( P_o \) to be underestimated. This factor may contribute to the disparity between the forces reported by Hanson and Lennerstrand (1977) and Bach-y-Rita and Ito (1966).

The anatomy of the levator and superior rectus muscles are quite similar in humans and rats, with each originating from the annulus of Zinn, the levator muscle being medial and external to the superior rectus muscle. The superior branch of the third nerve innervates both muscles, coming into the underside of the superior rectus and through its upper surface to enter the levator muscle. In its anterior two-thirds, the levator muscle lies directly above the superior rectus muscle but without significant attachments to it. Orbital fat is interposed between them along the posterior third of each muscle. In humans, there is evidence that co-contraction of the superior rectus has little effect on the force output of the levator muscle. The force generated by the levator muscle has been measured in patients by having them look upward maximally (volitional contraction of the adjacent extraocular muscle that might conceivably have contributed to the force output was eliminated) (Frueh and Musch 1990). In patients with ptosis (drooping upper eyelid) caused by levator muscle myopathy and with normal elevation of the eye (indicating a normal superior rectus muscle), the force of the levator muscle was as small as 40–50 mN, while the normal range of levator force was 370–1,000 mN (Frueh and Musch 1996). Therefore the superior rectus muscle would have contributed little to the forces recorded. In the present study, co-contraction of the adjacent extraocular muscle that might conceivably have contributed to the force output was eliminated by exciting the anterior two-thirds of that muscle.

In preliminary experiments, where the eye was not enucleated, we noted that the eye retracted \( \sim 1.8 \) mm on stimulation of the third nerve. We suspected that the retractor bulbi muscles were contributing to the force output since the anterior end of the superior portion of these muscles was in contact with the superior rectus muscle. Posterior to the globe, orbital fat is interposed between the retractor bulbi muscles and the rectus muscles. Therefore these muscles were not only separated from the superior rectus muscles but were severed from the globe, allowed to retract, as were the other extraocular muscles, and the globe removed. These procedures would have negated any effects of co-contraction of surrounding muscles contributing to the recorded force of the specific extraocular muscle attached to the force transducer.

There was considerable variation in fiber length \( (L_o) \) within each muscle (Fig. 1). The superior rectus muscle contains multiply innervated slow tonic fibers while the levator does not (Harker 1972). Analysis of serial sections of the orbital surface layer of the superior rectus muscle of the rabbit indicated that the population of multiply innervated fibers were longer than the more numerous singly innervated fibers (Davidowitz et al. 1977). This may explain why the average fiber length of the superior rectus muscle is greater than that of the levator muscle.

The \( sP_o \) of a skeletal muscle is more accurately described when the estimated muscle CSA is derived from the use of average fiber length \( (L_o) \) as opposed to the commonly but incorrectly used optimum muscle length \( (L_e) \). Under appropriate conditions for temperature \((25^\circ \text{C})\) and muscle stimulation in vitro (i.e., use of a power amplifier to increase and sustain current intensity sufficient to produce a maximal isometric tetanic contraction), \( P_o \) of the extensor digitorum longus (EDL) muscle of an adult male rat (muscle mass: 240 mg; \( L_o \); 37 mm), will usually exceed 3,000 mN (Gregorcic et al. 2000). Assessments of \( sP_o \) using \( L_e \) (for the calculation of CSA) instead of \( L_o \), would yield a value of 552 kN/m\(^2\) for the rat EDL muscle. When \( L_o \) is used instead of \( L_e \) for the calculation of muscle CSA, the \( sP_o \) is \( \sim 243 \) kN/m\(^2\).

Similarly for the extraocular muscles, assessment of \( sP_o \) based on CSA values derived from the incorrect use of \( L_o \) would yield \( \sim 724 \) kN/m\(^2\) for the levator muscle and \( \sim 622 \) kN/m\(^2\) for the superior rectus muscle. However, when the \( sP_o \) is calculated using a muscle CSA derived from the calculated \( L_o \) values, the \( sP_o \)’s measured in situ are \( \sim 275 \) kN/m\(^2\) for the levator muscle and 283 kN/m\(^2\) for the superior rectus muscle. These \( sP_o \) values for extraocular muscles are therefore consistent with those reported for limb skeletal muscles of rats and mice (Brooks et al. 1995; Devor and Faulkner 1998; Lynch et al. 1999; Van der Meulen et al. 1997). The \( P_o \) and \( sP_o \) values for limb skeletal muscles of the rat are similar whether they are investigated in situ or in vitro (Brown and Hasser 1996). The much lower \( sP_o \) values for extraocular muscles examined in vitro compared with in situ is therefore difficult to explain. The difference reflects, at least in part, how easily the origin of these muscles can be damaged during the intricate dissection procedures since there is no tendon at the origin of the extraocular muscles. Another contributing factor that could explain the disparity between the specific forces obtained under the different experimental conditions relates to whether electrical field stimulation (as opposed to direct stimulation of the nerve) can recruit all fibers within extraocular muscles, especially when the orientation of the fibers may not be optimal. Our results indicate that maximum \( P_o \) was only obtained (in situ) when the extraocular muscles were orientated \( 45^\circ \) above the horizontal, an orientation difficult to mimic in vitro. This fact coupled with the often atypical fiber architecture within extraocular muscles, provides some explanation as to why this disparity in \( sP_o \) exists under the two different experimental conditions.

Our findings reveal that the force output of intact extraocular muscles differs greatly depending on the mode of testing. In situ evaluation preserves extraocular muscle function such that \( sP_o \) values are similar to those for limb skeletal muscles. However, most skeletal muscles develop the same forces in situ and in vitro, whereas extraocular muscles generate far less force in all studies performed in vitro. Although in vitro evaluation of extraocular muscle contractility will continue to reveal important information about these very much understudied muscles (Campbell et al. 1999; Lynch et al. 1994), the lower \( sP_o \) values of these preparations should be recognized as being significantly less than the true potential for these muscles. We should no longer view extraocular muscles as intrin-
BROWN AND MARÉCHAL. Complexity of age-related change in skeletal muscles but every bit their equal in specific force.

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