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Characteristics of sarcomere length operating ranges in the rat lumbar spine extensor muscles: comparison to human

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SUMMARY

Proper coordination and function of muscles is required to move and stabilize the spine. However, due to difficulty in sampling, few empirical data exist regarding the specific structural and architectural characteristics of spine muscles in humans. The use of animal models is an alternative; but comparisons between animal and human spine muscles are lacking. Therefore, ten adult male Sprague-Dawley rats were euthanized, skinned and immersion-fixed in formalin; six rats in a neutral spine position and four rats in a fully flexed spine position. Longissimus, iliocostalis and multifidus muscles were excised, individual fascicles were dissected and sarcomere lengths were measured via laser diffraction. Results demonstrated that mean sarcomere lengths in the neutral position were 2.29 μm, 2.44 μm and 2.44 μm for the multifidus, longissimus and iliocostalis, respectively. In the fully flexed posture, sarcomeres were lengthened to 2.79 μm, 2.92 μm and 2.91 μm, respectively. In the neutral spine posture, these muscles were closer to optimal length compared to those previously measured in humans. In the fully flexed posture, these muscles exhibited similar relative lengths (on the descending limb of the force-length relationship) compared to those previously modeled in humans. In summary, rat spine extensor muscles have longer sarcomeres, relative to optimal length, in the neutral posture compared to human spine extensor muscles. However, in the fully flexed spine position, both rat and human spine muscles act on the descending limb of the force -length relationship, all at similar relative lengths. These data provide a context to guide the use of rat spine muscles as a model to understand structural and functional characteristics in the human.

Key words: Muscle – Spine – Multifidus – Longissimus – Iliocostalis – Sarcomere – Animal model

INTRODUCTION

Muscles are necessary to move and stabilize the spine, as well as to protect against low back injury and pain. However, our fundamental understanding of specific characteristics of the spine muscles is very limited. This is largely due to a lack of measurement capability. Electromyography can be used to measure muscle recruitment and activation (e.g. Waters and Morris, 1970; Zetterberg et al., 1987; Moseley et al., 2002), and medical ima-

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ging (in particular MRI) can be used to measure muscle volumes and estimate magnitudes of fatty infiltration (e.g. Marras et al., 2001; D'hooge et al., 2012; Urrutia et al., 2018). However, true muscle architectural parameters such as physiological cross-sectional area and normalized fibre length cannot be calculated from medical images due to a lack of sufficient resolution necessary to measure sarcomere lengths (Brown and Gerling, 2012). Recent techniques have been developed to measure sarcomere lengths in vivo in humans (Llewellyn et al., 2008; Young et al., 2017), but these methods are invasive and very expensive, they only sample from a small number of sarcomeres, and their accuracy/utility is questionable. More detailed architectural and structural measures have been made from cadaveric donors and surgical patients (Ward et al., 2009). However, these results are limited/ biased to older individuals or only those with pain/ deformity great enough to require surgical intervention. Therefore, these data are of questionable representation of young healthy individuals and may not characterize normal healthy spine muscle function.

Animal models are an alternative. Small rodent models are widely used to study spine function (Alini et al., 2008). However, these are mainly dedicated to investigations of the intervertebral disc and/or pain responses. Recent research has explored various aspects of the spine musculature and its relationship to spine pathology using rat (Maas et al., 2018; Zwambag et al., 2018) and mouse (Gsell et al., 2017; James et al., 2018) models. However, how well these rodent muscles represent human muscles is not clear. Previous work has demonstrated that the abdominal wall muscles, also very important for moving, stabilizing, protecting and rehabilitating the spine, show remarkable similarities between rat and human (Brown et al., 2010). In particular, relative sarcomere lengths amongst the four abdominal muscles were strikingly similar between rats and humans. Sarcomere length distributions and organization have been shown to be quite interesting in human lumbar spine extensor muscles; in particular, the muscles have short sarcomere lengths in the neutral standing posture and reach optimal length in the approximate mid-flexed spine posture (Zwambag et al., 2014). It is well known that sarcomere lengths dictate the relative ability of muscles to generate force (Gordon et al., 1966), and therefore knowledge of muscle sarcomere length operating ranges is necessary to understand how muscles generate force throughout their ranges of motion (Burkholder and Lieber, 2001). Thus, the current work was designed to study the organization of sarcomeres in spine muscles of Sprague-Dawley rats to determine if they demonstrate similar characteristics to human spine muscles.

MATERIALS AND METHODS

Ten adult male Sprague-Dawley rats (mean (± SD) mass of 289 g ± 22.4) were euthanized via carbon dioxide inhalation. This number of animals is similar to many other studies conducted in this field (e.g. Bodine et al., 1982; Holewijn et al., 1984; Brown et al., 2010; Alpernin et al., 2015). Only male rats were studied as there is no scientific evidence nor clear hypothesis to suggest that male and female sarcomere length operating ranges would be different for these muscles. The procedures were approved by the University of Guelph Animal Care Committee. The rats were immediately skinned and immersed in a 10% formalin solution (Protocol 10% buffered formalin, Fisher Scientific, Mississauga ON, Canada) for a length of time between 72 and 120 hours (Sacks and Roy, 1982), and placed in either an anatomical position with a neutral spine (six rats), or a position with a fully flexed spine (four rats). After being fixed, the rats were rinsed in phosphate-buffered saline (PBS tablets, BioShop Canada, Burlington ON, Canada) to remove any residual formalin. The minimum time of 72 hours was necessary to ensure that the muscles were completely fixed and would therefore maintain their sarcomere lengths after the muscles were dissected away from the bone.

The lumbar spine extensor muscles (iliocostalis, longissimus, and multifidus) were excised and divided based on different origins and insertions. Specifically, iliocostalis and longissimus fibres all inserted on the iliac crest and were distinguished based upon their origins on the tip of the transverse process (iliocostalis) and the accessory/transverse process (longissimus) of the 13th thoracic vertebrae to the 5th lumbar vertebrae; the excised multifidus originated on the spinous processes of the 1st lumbar (most cranially tested) to the 4th lumbar (most caudally tested) vertebrae and inserted on the mammary or accessory processes of the 3rd to 6th lumbar vertebrae. Fascicles were dissected from each muscle segment and sarcomere lengths were measured via laser diffraction (Lieber et al., 1984) using a 5mW diode laser (wavelength = 635 nm, beam diameter ≈ 1.4 mm; Lasaris DLSC-635S -5, Coherent Inc., Santa Clara CA, USA). The laser beam was projected through fascicles, and the resulting diffraction patterns were recorded by a 256 element linear photo diode (RL1210LGQ-711, PerkinElmer, Waltham MA, USA) and digitally converted to be displayed on a computer screen. A custom LabVIEW program (LabVIEW 2010, National Instruments, Austin TX, USA) was used to define the peaks of the first order diffraction patterns, and the sarcomere length was then calculated by the equation:

$$SL = \frac{n\lambda}{\sin(\tan^{-1}\frac{x}{h})}$$

where SL is the sarcomere length, n is the diffraction order, λ is the laser wavelength, x is half the distance between 1st order diffraction peaks, and h is the distance between the muscle fascicle and photo diode. A minimum of three sarcomere length measurements were obtained from each muscle segment and then averaged for that segment. This laser diffraction technique is considered the gold standard for measuring relatively large populations of sarcomeres within a muscle, and has been used in both live and cadaveric human and animal studies (e.g. Lieber et al., 1984; Tamai et al., 1989; Lieber et al., 1994; Brown et al., 2010; Vaz et al., 2012; Zwambag et al., 2014)

Muscle strain between the neutral and flexed postures was calculated as:

$$Strain = \frac{SL_{flexed} - SL_{neutral}}{SL_{neutral}} * 100$$

Sarcomere lengths measured here, as well as the measured and modeled neutral and flexed sarcomere lengths reported in Zwambag et al. (2014), were additionally normalized to the estimated optimal sarcomere length for each specific species: rat 2.32 to 2.52 μm, human 2.64 to 2.84 μm, calculated as the range from twice the length of the actin filaments (from Burkholder & Lieber, 2001) to this length plus the width of the myosin bare zone (0.16 μm; Sjostrom and Squire, 1977), and assuming a z-disk width of 0.1 μm (Walker and Schrodt, 1974).

RESULTS

Measured sarcomere lengths in the neutral and flexed spine positions are shown in Table 1. Longissimus and iliocostalis had the same mean sarcomere length in the neutral spine position and almost identical strains from this reference position when fully flexed (19.7% strain for longissimus and 19.3% strain for iliocostalis). Mean multifidus strain was measured to be 21.8%. The sarcomere length ranges are displayed relative to the predicted rat force-length relationship in Fig. 1.

A comparison of sarcomere length ranges between the measured values for rat here and the measured (neutral) and modeled (flexed) values for human (Zwambag et al., 2014), normalized to

Table 1. Mean ± SEM sarcomere lengths (μm) measured in the neutral and flexed spine positions

	neutral	flexed
multifidus	2.29 ± 0.02	2.79 ± 0.01
longissimus	2.44 ± 0.02	2.92 ± 0.02
iliocostalis	2.44 ± 0.02	2.91 ± 0.02

Table 2. Measured (rat neutral and flexed; human neutral) and modeled (human flexed) sarcomere lengths shown as percentages relative to optimal length (rat 2.32 to 2.48 μm; human 2.64 to 2.80 μm).

	Rat		Human	
	neutral	**flexed**	**neutral**	**flexed**
multifidus	-1.3	12.5	-10.6	11.1
longissimus	0.0	17.7	-8.0	16.4
iliocostalis	0.0	17.3	-8.7	15.4

Negative values indicate sarcomeres on the ascending limb of the F-L relationship (percentage shorter than optimal), values of zero indicate optimal length, and positive values indicate sarcomeres on the descending limb of the F-L relationship (percentage longer than optimal).

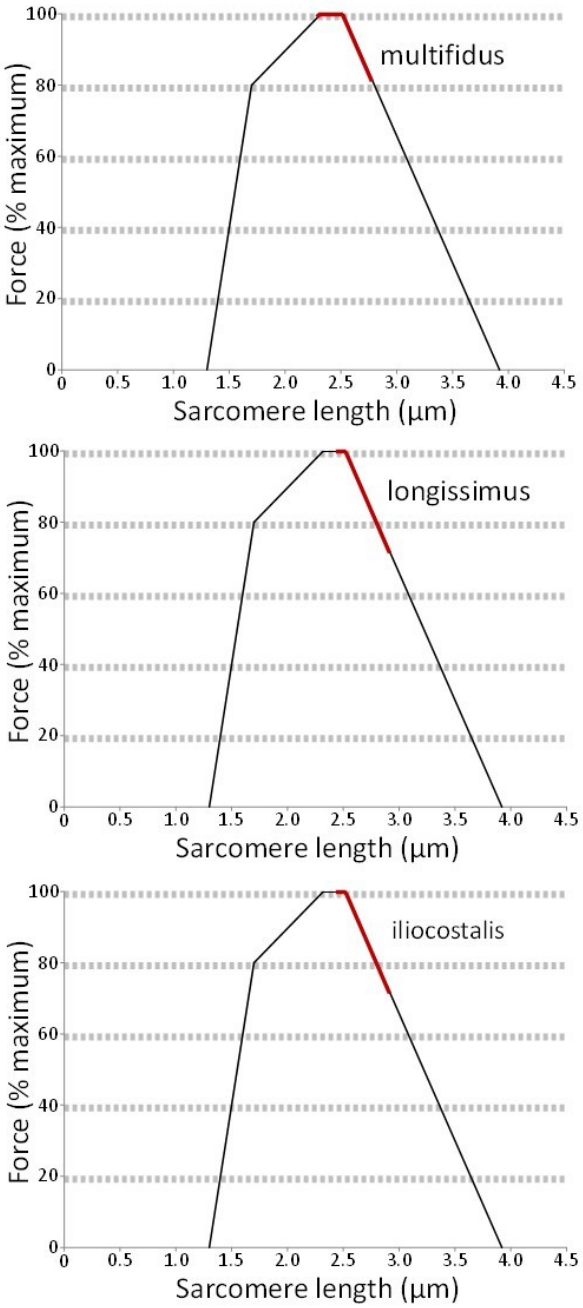

Fig 1. Measured sarcomere length operating ranges, shown in red on representative force-length relationship curves, of the rat multifidus, longissimus and iliocostalis muscles from neutral spine to flexed spine positions.

optimal length for both species, are shown in Table 2.

DISCUSSION

Rat lumbar spine extensor muscles have longer sarcomeres, relative to optimal length, in the neutral cadaveric posture compared to human spine extensor muscles. Rat multifidus, longissimus and iliocostalis all act at or slightly below optimal length in this position, whereas in humans all three muscles act on the ascending limb of the force-length relationship, approximately 8-11% shorter than optimal length. However, in the fully flexed spine position, both rat and human spine muscles act on the descending limb of the force-length relationship, all at similar relative lengths (multifidus 11-12% longer than optimal; longissimus and iliocostalis 15-17% longer than optimal).

These data demonstrate that the sarcomere length operating the range of spine extensor muscles is shifted to the right in rats compared to humans, such that both show the characteristic of a progressive decline in the ability to generate active contractile force, at ranges of motion at least beyond mid-flexion. However, in the neutral spine position, rats are optimally able to generate active force. This may be a fundamental requirement of the quadruped nature of rat ambulation. Geisler et al. (1996) noted that tonic activation of the spine extensors was required to maintain a quadruped posture and that locomotion required an additional clear phasic activation of these muscles. Further, significant activation was required during rearing and this activation was greatest when the spine was straightest (i.e. most upright). When rats flex their spines, their spine muscles lengthen to become progressively compromised in their ability to generate force. Actions that would require spine flexion, such as grooming, have also been demonstrated to require activation of the lumbar spine muscles (Geisler et al., 1996), but interestingly this activation is relatively lower (compared to upright rearing or peak phasic locomotion) in spine positions including latero-flexion (Geisler et al., 1996; pure flexion was not discussed in this paper).

When humans are in the neutral spine posture, their spine extensor muscles act on the ascending limb of the force-length relationship, and therefore have not yet reached optimal length to generate active force. Normal activities in this posture only require minimal activation and force generation to sufficiently stabilize the spine (Cholewicki and McGill, 1996). As humans begin to flex their spines, their muscles lengthen to reach optimal force generating capability near the mid-flexed posture. Lengthening toward optimal would make mechanical sense, as the lumbar extensor moment demand increases as flexion increases; however, decreasing force generating capability beyond mid-flexion would seem counterintuitive. Humans routinely flex their spines through a number of tasks of daily living, from sitting to lifting. In sitting, where the lumbar spine is normally flexed to approximately 70% of maximum (De Carvalho et al., 2017), these muscles would already be onto the descending limb of the force-length relationship and beyond optimal length for generating active force (Zwambag et al., 2014). When flexing further, for example to perform a lift from near ground level, the muscles are even more compromised in their ability to generate force yet the moment demand on the muscles is highest. This seeming contradiction is characterized by what is termed the flexion-relaxation phenomenon, where many spine muscles become electrically silent (and therefore generate no active force) as flexion approaches maximum. It had long been thought that in this situation the moment was supported by tension in the ligaments and intervertebral discs, but new data suggests that it is actually primarily supported by passive force being generated within spine muscles that have been stretched to these long lengths (Zwambag and Brown, 2020). Experimental data have shown the passive force in stretched rat spine muscles to be substantial (Zwambag et al., 2019), which might suggest that rats could also benefit from a similar mechanism. However, flexion-relaxation has never been examined in a rat or other quadruped. As noted earlier though, during rearing, rat latero-flexion requires significantly less spine extensor activation compared to upright rearing (Geisler et al., 1996), suggesting that a similar mechanism to flexion-relaxation could exist.

Sarcomere length determines where on the force-length relationship a muscle acts during contraction, and thus is a fundamental determinant of a muscle's ability to generate force (Gordon et al., 1966). Other important architectural determinants of force and moment generating capability, namely physiological cross-sectional area, normalized fibre length (Lieber and Friden, 2000) and moment arm lengths, respectively, have not been compared in the spine muscles between humans and rats. For a more complete understanding of the similarities and differences in how these species move and load their spines, these issues should be addressed in the future.

The human data, to which the rat sarcomere length operating ranges are being compared, were obtained from cadavers that were fixed by arterial perfusion with a 2.5% formalin solution as opposed to the immersion fixation in a 10% formalin solution used here. However, it is not expected that these differences would have any impact on the sarcomere lengths measured in the two studies. Further, the methods to measure sarcomere lengths were identical in the human study (Zwambag et al., 2014) and the current study.

In summary, rat and human lumbar spine extensor muscles are designed to generate maximal

force in the neutral and mid-flexed spine positions, respectively. In both species as the spine flexes, these muscles lengthen and eventually reach similar locations on the descending limb of the force-length relationship in full spine flexion, indicating similar relative force generating capabilities in this position. It is likely that both the similarities and differences in these sarcomere length-based characteristics are related to species-specific functional requirements of the muscles, and this idea needs to be further explored. The rat can serve as a useful model to explore aspects related to human spine extensor muscle function, especially toward the end range of flexion motion.

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