Swelling of sarcoplasmic reticulum in the periphery of muscle fibres after isometric contractions in rat *semimembranosus lateralis* muscle

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ABSTRACT

The decline in isometric force, swelling of sarcoplasmic reticulum and loss of desmin was measured in *semimembranosus lateralis* muscle of male Wistar rats immediately after a short series of brief (500 ms) maximal isometric contractions. For the active muscle, the series ended below (protocol A) and just over muscle optimum length (protocol AA). In one protocol, the muscle remained passive and was extended to lengths just over muscle optimum length (protocol P). After all experimental protocols, no loss of desmin was observed and sarcomere appearance was normal. Protocol A produced swelling (87%) of the sarcoplasmic reticulum but no decline in isometric force. Protocol AA produced larger swelling (147%) of the sarcoplasmic reticulum and an isometric force decline (<49%) at short muscle lengths. Swelling of sarcoplasmic reticulum was observed mainly in the periphery of muscle fibres. Protocol P did not result in swelling of the sarcoplasmic reticulum and isometric force decline. It is concluded that swelling of the sarcoplasmic reticulum in the periphery of muscle fibres after brief maximal isometric contractions is associated with muscle force and not muscle length.

Keywords desmin, force–length relationship, isometric contraction, muscle fibre, muscle optimum length, sarcomere, sarcoplasmic reticulum.

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Isometric contractions at long muscle lengths, if done often enough, will lead to overextension of some sarcomeres according to the non-uniform sarcomere hypothesis by Morgan (1990), and will resemble the structural changes seen after eccentric contractions (Wood *et al.* 1993). Eccentric contractions alter the appearance of sarcomeres by Z-band streaming (Fridén *et al.* 1981, Ogilvie *et al.* 1988, Wood *et al.* 1993) which was associated with a disruption of desmin, i.e. muscle injury (Lieber *et al.* 1996). The disruption of desmin represents an early manifestation of muscle injury during eccentric contractions (Lieber *et al.* 1996). Such injury has also been linked with structural and functional alterations of the sarcoplasmic reticulum (SR) (for a review see Byrd 1992) as well as a loss in calcium homeostasis indicated by crystalline structures in the SR and increase in volume density of SR (Fridén & Lieber

1996). For skeletal muscle, all these ultrastructural alterations are always associated with a decline in force production (Fridén *et al.* 1983, Lieber *et al.* 1991, Wood *et al.* 1993, Lieber *et al.* 1996).

In human skeletal muscle, a greater decline in force production was observed after a fatiguing protocol of isometric contractions around muscle optimum length (i.e. the length at which the muscle produces maximal active force) (Fitch & McComas 1985, Sacco *et al.* 1994). This observation maybe accounted for by muscle length or muscle force related changes to the SR. In rat *semimembranosus lateralis* muscle, a decline in force production was observed after a series of isometric contractions ending at 110–120% of the muscle optimum length. (Huijing *et al.* 1989, Willems & Huijing 1992). Such a series is essential to provide detailed information on the force–length relationship of skeletal muscle *in situ*

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(Willems & Huijings 1994). It is not known if the decline in force production in this skeletal muscle is related to an effect of long muscle length or muscle force. In addition, the decline in force production could be associated with a loss of desmin and/or structural changes in SR.

The goals of this study were therefore twofold. The first was to investigate whether the decline in force production in rat *semimembranosus lateralis* muscle immediately after a series of isometric contractions was related to muscle length or muscle force. A series of passive force–length measurements were carried out to analyse the effect of long length per se. The second goal of the studies was to relate the decline in force production to the loss of desmin (i.e. the appearance of sarcomeres) and ultrastructural alterations of the SR.

METHODS

Animals

Experiments were performed on *semimembranosus lateralis* muscle of young adult male Wistar rats. Animals were anaesthetized with an intraperitoneal (i.p.) injection of diluted (1:5) nembutal (80 mg kg⁻¹ body mass), and placed on a heated pad (35 °C) during surgery and experimentation. Supplementary i.p. injections of diluted (1:10) nembutal (20 mg kg⁻¹ body mass) were administered as needed. Animal care and experimental procedures were performed in accordance with animal welfare regulations and guidelines set forth by Dutch law. All measurements were carried out at room temperature (22 °C).

Rat *semimembranosus* muscle (SM) is a bi-articular skeletal muscle originating from the periost of the pelvis extending from the *tuber ischiadicum* to *ramus ossis ischii*. Within SM, the lateral head (SML) can be distinguished from the medial head (SMm) because of clearly separate insertions on the medial epicondyle of the tibia. SML has a unipennate architecture, albeit of a very low degree of pennation (Willems & Huijings 1992) and a relatively short external tendon plate at its distal end (estimated length being less than 4% of the active muscle optimum length (l_o), i.e. muscle length at which maximum active force is exerted).

Experimental handling before executing the experiments

A detailed description of the dissection procedure for SML has been provided elsewhere (Willems & Huijings 1992). In brief, the aim of the procedure is to obtain an *in situ* SML with an intact blood supply and innervating nerve which is isolated from the central nervous system. The tibia was removed from the femur by cutting the knee joint. Subsequently, a hole was drilled through

the epicondyles of the tibia and the tibia cut off just distally to the insertion of SML. The mass of the remaining part of the tibia was about 0.8 g. A kevlar thread (length about 90 mm, diameter 0.5 mm, 4% elongation at breakload of 800 N) was tied to the remaining part of the tibia through the hole. This part was attached to a strain gauge force transducer (stiffness 245 N mm⁻¹). The pelvis was fixed by clamping the *spina ischiadica* with a metal clamp. Muscle and remaining part of tibia were supported by a synthetic plate excluding effects of gravitational forces on functional characteristics. The force transducer was firmly mounted on a slide with a horizontal travel range of 56 mm. The total length of the line of origin of SML and SMm (entire SM, i.e. the distance between the origin of the most proximal fibre of SML and the origin of the most distal fibre of SMm) was marked by two needles inserted into the pelvis. Additional markers of copper wire (diameter 0.1 mm) were inserted in the muscle: one at the most distal end of the proximal fibre (proximal aponeurosis marker) and one at the most distal end of the aponeurosis (distal aponeurosis marker) (Fig. 1). The muscle was prevented from drying by applying paraffin oil.

Experimental protocol and experimental design

For determination of muscle geometry and isometric active length–force characteristics, the muscle was positioned at a desired length and a tetanic contraction of 500 ms duration was evoked by supramaximal stimulation of the distal end of the sciatic nerve using square wave pulses (duration 0.5 ms, amplitude 3 mA, frequency 100 Hz), through a pair of silver electrodes connected to a constant current source. Total force was measured while the complex of kevlar thread, tendon plate and muscle was photographed (Fuji 400 ASA colour reversal film, Canon T70 camera with a 100 mm macro lens, exposure time 1/60–1/125 s, diaphragm F11–F16) 400 ms after start of the stimulation, i.e. during the force plateau of the tetanus. The plane of the film was carefully aligned with the plane through the markers inserted into the muscle and the pelvis. For determination of muscle geometry and passive length–force characteristics, the muscle was positioned at a desired length and after 2 s passive force was measured while the complex was photographed. All measurements were executed at time intervals of 3 min to avoid potentiation or fatigue effects.

Based on a previous work (Willems & Huijings 1994) the length range between muscle active slack length (l_{mas} , i.e. muscle length at which active force approaches zero) and l_o is ≈ 21 –22 mm.

In four groups of muscles, all protocols (described below) started with three tetanic contractions at short

Figure 1 (a) Fibre arrangement of rat *semimembranosus lateralis* muscle and marker locations. (b) Geometry as derived from projected images of the muscle. Length variables measured were muscle length (l_m), length of the most proximal fibre (l_{fprox}) and length of the most distal fibre (l_{fdist}). Orientation angle (β_0) was calculated according to the law of cosines in an appropriate triangle. Note that the lower figure is not drawn to scale for a better representation of fibre locations within the muscle.

muscle lengths in order to estimate l_{mas} . The performance of the three contractions will be referred to as general treatment. Protocols were: (1) exposure to general treatment (control group (C), $n = 5$); (2) exposure to general treatment followed by a series of passive length–force measurements between estimated l_{mas} and ≈ 2 mm over l_o (P, $n = 6$); (3) exposure to general treatment followed by a series of brief isometric contractions between estimated l_{mas} and about 11 mm over l_{mas} (A, $n = 6$); or (4) exposure to general treatment followed by a series of brief isometric contractions between estimated l_{mas} and ≈ 2 mm over l_o (AA, $n = 6$). Rat body mass of C, P, A, and AA-group were 296.5 ± 1.5 , 300.2 ± 8.6 , 292.0 ± 4.6 and 294.5 ± 7.2 g (mean $\pm$ SD), respectively.

Passive or active length–force measurements in protocol 2, 3 and 4 will be referred to as experimental protocol P, A, and AA, respectively. After the general

treatment in P-, A- and AA-muscles, one isometric contraction was performed at 11 mm over estimated l_{mas} . This length will be referred to as control length and active forces measured at this length will be referred to as pre-values of active force (P-pre, A-pre and AA-pre, respectively). Based on previous work (Willems & Huijing 1994) active force is expected to be 50–60% of muscle optimum force. The series of passive length–force measurements was started at estimated l_{mas} , despite the fact that the muscle will be slack for several mm, followed by seven length increments of 1 mm, five of 2 mm, and seven of 1 mm. Passive measurements were terminated as the increase of origin–insertion length attains 24 mm relative to estimated l_{mas} . This is estimated to be just over l_o . Subsequently, one tetanic contraction was performed at control length again and active force measured will be referred to as post-value of active force (P-post).

The series of brief isometric contractions were started at estimated l_{mas} , followed by the same length increment protocol as in the series for the passive length–force measurements. Protocol A was terminated when control length was reached again. The active force measured during the last contraction will be referred to as post-value of active force (A-post). Protocol AA was terminated about 2 mm over l_o . Subsequently, force was measured at about 3 mm below (AA-post 1) and 3 mm (AA-post 2) over control length to check the decline in force production at various fibre lengths.

In between any measurement the muscle was allowed to recover at origin–insertion length corresponding to estimated l_{mas} . Force transducer signals as well as photography synchronization signals, were A-D converted and recorded by microcomputer (sample frequency 1000 Hz). Timing of stimulation in the active experiments, photography, A-D conversion of force values and synchronization signals of photography, and rest periods between measurements were controlled by a special purpose microcomputer.

Collection of morphological and structural data

Owing to practical problems the length of the line of origin of SMI (i.e. the distance between the origins of its most proximal and most distal fibre) could not be marked prior to the physiological part of the experiment. Careful anatomical examination of the length of the line of origin of SM after experimentation revealed that approximately the upper two-thirds of this length represented the length of the line of origin of SMI. Photographic images of SMI were projected onto a translucent screen (magnification $5\text{--}6 \times$ life-size). Estimates of muscle length (l_m) and lengths of the most proximal (l_{fprox}) and distal fibres (l_{fdist}) are defined in

Fig. 1, and were quantified using an ultrasound digitizer (Grafbar, Science Accessories, Southport, CT, USA) with an accuracy of 0.05 mm. Ignoring the curvature of fibres underestimates the length of these structures, but this was found to be maximally 3% at short fibre lengths (Willems & Huijing 1994). The orientation angle (β_o) was calculated according to the law of cosines in an appropriate triangle and is the angle between the line of pull and the line of origin of the muscle on the pelvis (Fig. 1). The pelvis was set at approximately the same position for all rats leading to about a similar β_o (Fig. 1) for different muscle groups (C: 71.6 ± 3.9 , P: 65.6 ± 3.0 , A: 75.5 ± 3.5 , and AA: 71.7 ± 5.3 deg, mean $\pm$ SD).

Post-experimental treatment

At the end of each experiment, remaining parts of the medial head of the *semimembranosus* were removed. For electron microscopical analysis, a muscle exposed to each of the four protocols was positioned with needles on a cork plate at a length ≈ 11 mm over l_{mas} . Subsequently, muscle specimens were fixed (length and diameter were ≈ 8 – 10 and 5 – 7 mm, respectively) in 2.5% glutaraldehyde in phosphate-buffered saline (PBS: 0.15 M NaCl, 0.01 M phosphate buffer, pH 7.2). Fixed specimens were washed in PBS and cut into small blocks (1×2 mm²) along the longitudinal axis of the muscle and then post-fixed in 1% osmium tetroxide in PBS, dehydrated in a graded series of acetone, and embedded in Polybed 812. Three tissue blocks from proximal and distal portions of each specimen were chosen at random and one block from each region was longitudinally oriented and sectioned into two halves. One half was used for transverse sectioning the other for longitudinal. Six semithin sections from each orientation (1 μ m) were stained with Toluidine blue. Sections were then viewed under the light microscope in order to determine select areas for analysis. Select areas were then cut in 50-nm thick longitudinal and transverse sections on an LKB Ultratome III. These sections were picked up on Formvar-coated copper grids and stained with uranyl acetate and lead citrate. Stained sections were viewed and photographed in a Jeol 12000 EXII electron microscope (Tokyo, Japan). In order to assess the overall ultrastructure, transverse and longitudinal sections were viewed ($\times 1200$).

Stereology

High-voltage electron micrographs of transverse sections were analysed quantitatively by means of a 168 point multipurpose test system according to Weibel (1980). Six ultrathin sections were obtained from each block, a grid square was chosen at random within each section and one micrograph was taken in each ran-

domly chosen grid square. The transparent multipurpose system was placed in the geometric centre of the micrograph. The total number of points overlying intermyofibrillar SR and SR membrane was recorded and the volume density of SR in transverse sections were estimated (Weibel 1980).

Immunohistochemistry

Muscles for immunohistochemical analysis were fastened to a wooden stick at ≈ 11 mm over l_{mas} using synthetic strips, and frozen in liquid isopentane (-80 °C) cooled by liquid nitrogen (-159 °C) and stored at -73 °C until analysis. Five-micron thick cryosections were stained with antibodies against desmin (monoclonal, mouse antihuman) for the structural integrity of the cytoskeletal network (Thornell *et al.* 1985). Antibody binding was visualized by the indirect peroxidase–antiperoxidase technique (Dakopatts, Copenhagen, Denmark) (Sternberger 1979).

Data processing

In each group the shortest muscle length at which any muscle force was found was determined by extrapolating a one-degree polynomial curve through results of the general protocol. This length was defined as the muscle active slack length (l_{mas}).

Data of experimental treatment AA were (least squares) fitted for individual muscles with a polynomial according to the equation:

$$F_m = A_0 + A_1 * l_m + A_2 * l_m^2 + A_3 * l_m^3 + \dots + A_n * l_m^n \quad (1)$$

where A_0 through A_n are constants selected in the fitting procedure. Using an analysis of variance (ANOVA) the degree of the polynomial which most adequately described a particular set of length–force data was selected (see Statistics below). The muscle length at which a maximum force occurred was defined as active muscle optimum length (l_o). Force values of AA-post 1 and AA-post 2 were compared with calculated pre-values (AA-pre 1 and AA-pre 2) using the best fitting polynomial. The relationship between l_m and l_{prox} and l_{dist} were (least-squares) fitted with a third-degree polynomial. At muscle control length, l_{prox} and l_{dist} were calculated and averaged. Pre- and post-values of active force were compared at similar average fibre lengths.

Statistical analysis

Analysis of variance (ANOVA) was used to select the orthogonal polynomial of the lowest degree that provided an adequate fit for a particular length–force data set (Snedecor & Cochran 1989). It should be noted that for each length–force curve of an individual muscle a

different degree may be selected to obtain a statistically optimal description of the data. ANOVA was used for analysis of l_{mas} , desmin loss and volume density of SR between groups. For each muscle, the difference between pre- and post-value of active force was expressed as a percentage change in active force. A one sample *t*-test ($P < 0.05$) was used to determine whether the percentual change was significantly different from zero, i.e. whether the experimental protocol significantly altered active force at control length. A Student *t*-test ($P < 0.05$) was used for comparison of highest values of average fibre lengths acquired during experimental protocol P and AA.

RESULTS

Typical examples of the series of passive and active isometric contractions

Figure 2 shows for P, A and AA-muscles typical examples of mean fibre length–muscle force data. After experimental protocol P and A, post-values of active force were similar to pre-values. Note that pre- and post-values were measured at similar mean fibre lengths (Fig. 2). Despite that fact, AA-post 1 and AA-post 2 were lower compared with calculated AA-pre 1 and AA-pre 2. The force value before experimental protocol AA (i.e. AA-pre) was similar to the force value during experimental protocol AA.

Muscle and fibre lengths

Table 1 shows values for a set of length variables for the experimental and control groups. The muscle active slack lengths were not significantly different between groups. The highest average fibre length at which isometric contractions were executed was significantly larger in muscles from the AA-group. The highest passive mean fibre length in muscles from the P group was not statistically different from the longest active mean fibre length in the AA group (Student *t*-test, $P < 0.05$). It is concluded that muscles of the P- and AA-group were exposed to lengths over muscle optimum length. In each group, there was no significant difference between the mean fibre lengths at pre- and

Figure 2 Typical examples of average fibre length and muscle force data of rat *semimembranosus lateralis* muscle during various experimental protocols and pre- and/or post-control contractions (see Methods). (a): datapoints represent force values acquired for experimental protocol P (●), P-pre (○) and P-post (□); (b): datapoints represent force values acquired for experimental protocol A (●), A-pre (○) and A-post (□); (c): datapoints represent force values acquired for experimental protocol AA (●), AA-pre (○), AA-pre 1 (*), AA-pre 2 (□), AA-post 1 (+) and AA-post 2 (■).

Table 1 Muscle active slack length (l_{mas}), highest passive fibre length (l_{fp}), highest fibre length of tetanic contraction (l_{fa}), fibre length at muscle optimum length ($l_{\text{f-lo}}$), pre-condition reference fibre length (pre- l_{fa}) and post-condition reference fibre length (post- l_{fa}) for experimental and control groups

Groups	l_{mas} (mm)	l_{fp} (mm)	l_{fa} (mm)	$l_{\text{f-lo}}$ (mm)	pre- l_{fa} (mm)	post- l_{fa} (mm)
Control	17.55 ± 1.16	—	15.85 ± 1.17	—	—	—
P	17.22 ± 0.54	38.41 ± 0.81	20.28 ± 0.65	—	20.28 ± 0.65	20.17 ± 0.50
A	17.41 ± 1.04	—	20.06 ± 1.34	—	20.06 ± 1.34	19.91 ± 1.31
AA1	17.74 ± 1.19	—	36.10 ± 3.01	33.70 ± 2.09	18.34 ± 2.11	18.43 ± 2.04
AA2	17.74 ± 1.19	—	36.10 ± 3.01	33.70 ± 2.09	22.71 ± 2.92	22.83 ± 2.53

See Methods for details on treatment of each group. Values are presented as mean ± SD.

post-values of active force. Thus the differences in active force values cannot be explained by differences in mean fibre lengths.

Isometric force

No experimental effect on isometric force could be shown for protocol P and A, as values of active force for P-post and A-post were not significantly altered compared with their pre-condition values (Fig. 3). Therefore, it is concluded that a series of passive force-length measurements ending just over muscle optimum length does not result in a subsequent force decline at low muscle length. However, experimental protocol AA yielded a significant decrease in force values of AA-post 1 and AA-post 2 compared with their pre-condition values. For AA-post 1 and AA-post 2, changes were $48.6 \pm 22.0\%$ (range 23.4–81.7), and $20.3 \pm 10.7\%$ (range 7.3–36.8), respectively. In contrast, force decreases were similar at the two reference lengths: for AA-post 1: 1.14 ± 0.66 N, and for AA-post 2: 0.86 ± 0.51 N). It is concluded that a series of brief isometric contractions ending just over muscle optimum length results in a substantial decline in subsequent force production at low muscle lengths.

Muscle ultrastructure

The percentage of muscle fibres showing loss of staining for desmin was not significantly affected by the brief isometric contractions (C: $4.95 \pm 3.00\%$; P: $3.72 \pm 3.64\%$; A: $5.32 \pm 2.59\%$; AA: $4.08 \pm 3.22\%$). The arrangement of sarcomeres appeared normal. Muscles exposed to A and AA protocols showed swollen vesicles, predominantly located at the periphery of the muscle fibres (Figs 4 and 5) and representing swollen SR (Fig. 6). Note that not all fibres show such ultrastructural alterations (Fig. 4). The volume density of the SR was significantly increased in the muscle exposed to protocols A and AA

Figure 3 Active force produced by rat *semimembranosus lateralis* muscle before and after various experimental protocols. Values for pre (□) and post (■) for various protocols. See Methods for abbreviations for various protocols. * indicates that the active force values after protocol AA (values for AA-post 1 and AA-post 2 contractions) were significantly different from their respective pre-values ($P < 0.05$).

(Fig. 7). Volume density was highest after protocol AA. The volume density following protocols A and AA was increased by 87 and 147%, respectively. Note that the increases for the volume density of SR are regarded as representative for the volume density of SR in large part located in the periphery of muscle fibres. It is concluded that a series of brief isometric contractions at lengths just over muscle optimum length does not affect the cytoskeletal network but results in swelling of SR in the periphery of muscle fibres.

Figure 4 Electron micrograph showing a cross section of several muscle fibres exposed to protocol AA (see Methods). Note the abundance of vesicles in the periphery of muscle fibres representing swollen SR (bar = 7.4 µm).

DISCUSSION

This study demonstrated swelling of the SR in the periphery of muscle fibres of rat *semimembranosus lateralis* muscle immediately following a series of brief maximal isometric contractions. Desmin was not affected by the brief isometric contractions. Accordingly, the ultrastructural appearance of sarcomeres was normal. The series of brief isometric contractions ending just over

Figure 5 Electron micrograph showing a longitudinal section of several muscle fibres after protocol AA showing the distended SR located peripherally (bar = 7.3 µm).

Figure 6 Electron micrograph showing a longitudinal section of several myofibrils after protocol AA with extremely swollen and disrupted SR containing remnants of membrane components. Note normal arrangement of sarcomeres (magnification calculated on the basis of a thick filament length of 1.59 µm, van Lookeren Campagne *et al.* 1988).

muscle optimum length produced the most extensive swelling of the SR simultaneous with a decline in force production at low muscle lengths. The swelling of the

Figure 7 Volume density of SR following various experimental protocols. Note that short (protocol A) and long (protocol AA) series of isometric contractions increase the volume density of SR with a larger increase after protocol AA. * indicates a significant difference between protocol A and control, ** indicates a significant difference between protocol AA and control, *** indicates a significant difference between protocol A and AA ($P < 0.05$, ANOVA).

SR was associated with the active forces at the various muscle lengths and not muscle length. We do not know whether the swelling of the SR following the series of contractions at lengths up to just over muscle optimum length was initiated by the contractions early in the series. Muscles exposed to such contractions also showed swollen SR (protocol A) but to a lesser extent. In addition, the protocol with muscles exposed to the series of isometric contractions just over muscle optimum length took 35 min longer than the group of muscles exposed to a shorter series of isometric contractions. Because Baracos & Goldberg (1986) have shown that resting the muscle at relatively short lengths activate proteolysis, we cannot exclude the possibility of an effect of proteolysis on the observed force decrements. In addition, we cannot exclude the possibility that the decline in force production has been present just before muscle optimum length was reached. However, these questions were not included in the major goals of the present study.

Swelling of sarcoplasmic reticulum

Swelling of the SR has been observed in the middle gluteal muscle of horses after exercise to fatigue with the largest increases at high intensities of exercise (McCutcheon *et al.* 1992). At such high intensities it was shown that the initial rate and maximal capacity of Ca^{2+} uptake of isolated SR were depressed (Byrd *et al.* 1989). These observations suggest that, in response to exercise, swelling of the SR alters its functional capacity. In human skeletal muscle, a depression in the capacity of Ca^{2+} uptake of the SR after exercise was associated with a marked decline in maximal voluntarily contractile strength and increase in half relaxation twitch time (Gollnick *et al.* 1991). The fact that in our study more swelling of the SR was observed after a series of isometric contractions ending at long muscle lengths might be linked with increased relaxation times of skeletal muscles at high lengths (Pagala 1980). Although direct evidence of swelling of SR and dysfunction has not been demonstrated, it is tempting to link the decline in force production with extensive swelling of the SR. In addition, the swelling has to be of a certain amount to relate them to the decline in force production. The present data suggest that force production of sarcomeres in the periphery of muscle fibres is substantially diminished following short maximal isometric contractions just over muscle optimum length. Our study shows that the swelling of the SR in rat *semimembranosus lateralis* muscle represents a true physiological response of SR following a series of maximal isometric contractions.

The question what causes swelling of the SR in the periphery of muscle fibres is not an easy one to answer.

Several factors may play a role. First, any alteration in the uptake and release mechanisms of ions between the myoplasm and the SR could create an ionic imbalance. For example, the release of Ca^{2+} from the SR occurs with an uptake of magnesium and potassium (Somlyo *et al.* 1981) of which the counter-transport during subsequent Ca^{2+} uptake might be confined. An ionic imbalance between the inside of the SR and the myoplasm forces the SR to serve as an osmotic basin. It swells because of an inward movement of water. It is interesting to note that during 8 s of 100 Hz stimulation of a single muscle fibre, a spatial gradient of Ca^{2+} developed, with a greater Ca^{2+} near the edges of the fibre than in the central part (Westerblad *et al.* 1990). Such spatial gradients, if present in whole muscle, could result in heterogeneous functioning of peripheral and central located SRs. Second, it is likely that peripheral SR is more susceptible to swelling for the following reason: in a skeletal muscle during force production, the region between adjacent muscle fibres is involved in transmission of force in a transversal direction and exposed to shear forces (e.g. Huijing *et al.* 1998), having a damaging effect on the sarcolemma which is likely to be muscle length dependent. It should be noted that rat *semimembranosus lateralis* muscle contains a heterogeneity of fibre mean sarcomere lengths in its muscle fibres (Willems & Huijing 1994). In this muscle at muscle optimum length there are fibres located on the descending limb of their length–force relationship based on their fibre mean sarcomere length. Therefore, it is hypothesized that damage (i.e. swollen SR) is related to very high sarcomere lengths at high muscle length in those fibres attaining their optimum length at low muscle lengths. A direct functional consequence of swollen SR would be a shift of muscle optimum length to higher muscle length, a shift observed following sustained isometric contractions (Huijing & Baan 1995, Huijing & Roszek 1996) and injury (Jones *et al.* 1997). Swelling of the SR may then result from damage to sarcolemmal membranes and the resulting imbalances of the Ca^{2+} homeostasis. It is tempting to speculate that swelling of the SR counteracts or minimizes the potential negative effects of any ionic imbalance and/or the potential damaging effect of successive large muscle forces. In addition, malfunctioning of the SR due to any mechanical damage as a result of the swelling may lead to enlarged concentrations of Ca^{2+} and enhance potential negative effects. Interestingly, a loss of SR membrane integrity was recently observed following eccentric contractions (Yasuda *et al.* 1997).

Malfunctioning of the SR may expose the muscle fibres to enlarged Ca^{2+} concentrations. Interestingly, swelling of SR vesicles during Ca^{2+} uptake causes spontaneous Ca^{2+} release (Beeler 1983). It is known that increased Ca^{2+} concentrations results in activation

of proteases and phospholipases involved in myofibrillar degradation (Armstrong 1990). The swelling of the SR is known to occur fast, within 15 min in intact amphibian skeletal muscle maintaining normal appearance of the sarcomeres (Duncan 1989). It precedes myofilament damage (Duncan 1989) and this damage resembles the damage observed with elevated Ca^{2+} levels (Duncan 1978) and following eccentric contractions (Armstrong *et al.* 1983). Duncan (1989) suggested a role of the SR in initiating myofilament damage. Interestingly, the force decrements immediately following eccentric contractions are due in large part to excitation–contraction coupling failure (Balnave & Allen 1995, Ingalls *et al.* 1998). It is hypothesized here that an early physiological response to the cascade of events leading to muscle injury and muscle soreness is swelling of the SR. Such a hypothesis was proposed by Byrd (1992). Specifically type 2B fibres are damaged predominately as a result of eccentric contractions (Fridén *et al.* 1983, Fridén & Lieber 1992). Fibre typing of rat *semimembranosus* muscle revealed that 60% consists of type 2B, 35% of type 2A and 5% of type 1 fibres (van Lookeren Campagne *et al.* 1988). It is suggested that further research in rat *semimembranosus lateralis* muscle should focus on ultrastructural damage in relation to fibre type.

In sum, a series of short maximal isometric contractions of rat *semimembranosus lateralis* muscle ending just over muscle optimum length results in a force decline at shorter muscle lengths and is associated with swelling of SR in the periphery of muscle fibres. The effect is attributed to the active force at various muscle lengths and not to muscle length. Future research should focus whether damage can be detected to the sarcolemma or SR.

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