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The effect of sarcomere length on triad location in intact feline caudofemoralis muscle fibres

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Summary

The location of triads within a mammalian skeletal muscle sarcomere has traditionally been defined as 'at the A-I junction'. We attempted to verify this statement by examining systematically the location of triads within the sarcomere over the physiological range of sarcomere lengths. This study was conducted using intact feline muscle fibres from caudofemoralis – an exclusively fast-twitch muscle from the hindlimb. Our results in intact fibres indicate that the distance between the Z-band and triad (ZT) is relatively constant over the range of sarcomere lengths (SLs) examined in this study (1.8–3.4 μm). The slope between ZT and SL was measured to be 0.06 ± 0.01 ($r = 0.36$, $p < 0.001$) while the slope between the M-line to triad distance (MT) and SL was measured to be 0.44 ± 0.01 ($r > 0.9$, $p < 0.001$). The mean ZT was 0.52 ± 0.07 μm, which corresponds to a triad location approximately halfway along the thin filaments. These results do not support the traditional statement regarding triad location. Nor do these results support a similar recent study conducted using chemically skinned muscle fibres from rat extensor digitorum longus (also a homogeneously fast-twitch muscle of the hindlimb), in which a slope of 0.25 was observed between ZT and SL ($r > 0.9$, $p < 0.01$). These results are, however, in qualitative agreement with results using intact fibres from fast-twitch rat semitendinosus. Based upon known morphology, we suggest that the only structure supporting triad position is the SR itself, and that a non-homogeneous distribution of the SR within the sarcomere might be responsible for maintaining triad location near the mid-region of the thin filaments. We also suggest that there might be optimal design reasons for locating the triads at the mid-region of the thin filaments.

Introduction

Excitation–contraction (E–C) coupling in skeletal muscle involves many critical steps, from propagation of the muscle action potential to the movement of troponin/tropomyosin to uncover the binding sites on actin and formation of cross-bridges. One of the central steps in E–C coupling is the release of calcium ions from the terminal cisternae of the sarcoplasmic reticulum (SR; see Franzini-Armstrong, 1994, for review). Muscle action potentials travel along the sarcolemma and down the transverse tubules to the triads, the point of contact between the transverse tubule system and the SR. Calcium stores in the terminal cisternae are then released into the sarcoplasm, diffusing throughout the half sarcomere into which they were released. This calcium then binds to troponin-C on the thin filaments to initiate cross-bridge cycling.

In amphibian muscle, triads are located at the Z-band at one end of the thin filaments (Smith, 1966; Eisenberg, 1983; Franzini-Armstrong, 1994). In contrast, mammalian triads are located away from the Z-band at a location usually referred to as 'at the A-I junction' (Smith, 1966; Eisenberg, 1983; Franzini-Armstrong, 1994). Only two studies have examined mammalian triad location at different sarcomere lengths to test the accuracy of this statement, both of which utilized fast-twitch muscles of the rat hindlimb (Ariano *et al.*, 1973). Bartels and coworkers (1979) used intact fibre bundles from rat semitendinosus, and qualitatively observed that the distance between the Z-band and triad was invariant over a sarcomere length range of 2.0–3.7 μm. Takekura and coworkers (1996) examined this same relationship in chemically skinned fibres from rat extensor digitorum longus (EDL) muscle. In contrast to Bartels and coworkers, Takekura and colleagues found that the distance between the Z-band and triad increased significantly as a sarcomere lengthened, with an increase of approximately 1 μm over a range of sarcomere lengths from 1.0–5.0 μm. Based on this observed movement, they concluded that triads were connected structurally at least in part to the thick filaments. The purpose of our

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study was to examine the relationship between triad location and sarcomere length in the more physiologically relevant whole-muscle preparation to test these contradictory findings.

Materials and methods

Caudofemoralis (CF) muscles from the hindlimbs of three female cats (2.8–3.0 kg) were used for this study. All three muscles had been used previously *in vivo* in a separate study (Brown & Loeb, 1998). CF was chosen because it is known to be composed homogeneously of fast-twitch muscle fibres (Ariano *et al*., 1973; Brown *et al*., 1998), similar to rat semitendinosus and rat EDL. Animals were perfused with 1.5 l fixative under deep anaesthesia (sodium pentobarbital; adequate anaesthetic level determined by absence of a pedal reflex) while holding CF near (± 20%) its maximum anatomical length. CF length was maintained by clamping the distal tendon to the surrounding tissue after positioning the animal for perfusion. Perfusate consisted of 2% paraformaldehyde, 1% glutaraldehyde and 0.1 M sucrose in 0.1 M phosphate buffer (pH 7.4). Following perfusion, the muscles were excised and stored in 0.5 l of fixative overnight.

Small samples from various parts of each muscle were taken for further processing. Samples were post-fixed with 1% OsO_4 and embedded in Epon. Thin (60 nm) longitudinal sections were cut and stained with uranyl acetate and lead citrate. The stained sections were viewed with a transmission electron microscope at 75 kV. Electron micrographs were taken at magnifications ranging from × 4000–12 000.

Distances were measured on the micrographs. Three distances were of primary interest: mid Z-band to mid-triad (ZT), M-line to mid-triad (MT) and sarcomere length (SL). To calculate these three distances, three measurements were taken: the mid-triad to mid-triad distance (TT) for a pair of triads and SL of the two sarcomeres adjacent to the triad pair (SL_1 and SL_2; note that occasionally only one adjacent sarcomere was visible and so only one SL was measured; see Fig. 1). SL was calculated as the mean of SL_1 and SL_2, while ZT and MT were calculated from the following formulas:

$$ZT = \left(\frac{SL - TT}{2}\right) \quad (1)$$

$$MT = \left(\frac{TT}{2}\right) \quad (2)$$

To account for shrinkage caused by the fixation and embedding process, scaling factors were chosen to make the A-band 1.6 μm long (Herzog *et al*., 1992).

Results

SL and TT distances were measured from at least 45 sarcomeres in each of the three muscles. While SL from the pooled data ranged between 1.8 and 3.4 μm, within each muscle the range was ~1.0 μm. Assuming that optimal sarcomere length (SL_0) is 2.4 μm (Herzog *et al*., 1992), the sarcomere lengths observed here range from approximately 0.75 to 1.4 SL_0, which covers almost the entire physiological range of motion for many feline (Brown *et al*., 1996), human (Cutts, 1988) and rabbit (Dimery, 1985) skeletal muscles. Longitudinal sections of four sarcomeres with lengths ranging from 2.1–3.0 μm are shown aligned along their Z-bands in Fig. 2. ZT and MT were plotted against SL for the pooled data (Fig. 3) with sarcomere length expressed in units of both μm and SL_0. A linear regression analysis between ZT and SL resulted in a slope of 0.06 ± 0.01 (slope estimate ± SE, $r = 0.36$, $p < 0.001$). The corresponding best-fit line is plotted along with the data in Fig. 3. If sarcomere lengths longer than the maximal anatomical length of CF are ignored (i.e. $> 1.2\ SL_0$ or approximately 2.9 μm; Brown *et al*., 1996, 1998), the slope is reduced to a non-significant 0.02 ± 0.02 ($r = 0.07$, $p > 0.40$). Similar analysis of MT v. SL produced a slope of 0.44 ± 0.01 ($r > 0.9$, $p < 0.001$) for the combined data and a slope of 0.48 ± 0.02 ($r > 0.9$, $p < 0.001$) for SL < 1.2 SL_0. The mean ZT over the entire SL range examined in this study was found to be 0.52 ± 0.07 μm (mean ± SD), halfway along the 1.1 μm thin filament (Herzog *et al*., 1992).

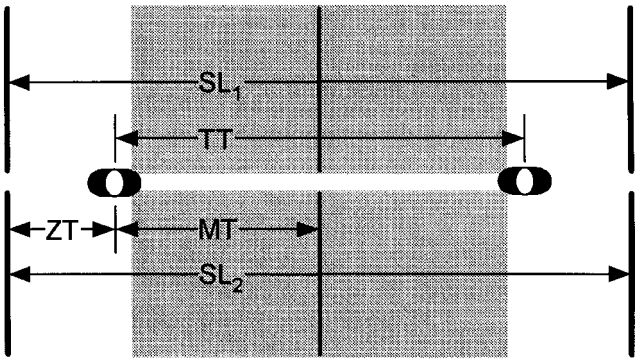

Fig. 1. Schematic of two sarcomeres and a triad pair between them. ZT, distance between the Z-band and triad; MT, distance between the M-line and triad; TT, distance between a pair of triads; SL_1 and SL_2, sarcomere lengths of the two adjacent sarcomeres.

Discussion

The main finding of this study was that triad location in relation to the thin filaments was relatively constant over the physiological range of sarcomere lengths. Traditionally, triad location in mammalian skeletal muscle has been described as 'at the A-I junction' (Smith, 1966; Eisenberg, 1983; Franzini-Armstrong, 1994), suggesting that triads should move away from the Z-band as a sarcomere is lengthened. While we did observe movement away from the Z-band, the slope of ZT v. SL required to support the above statement is 0.5, well above our calculated slope of 0.06. In fact, we only observed triads at the A-I junction when SL was near 2.6–2.7 μm (Fig. 2).

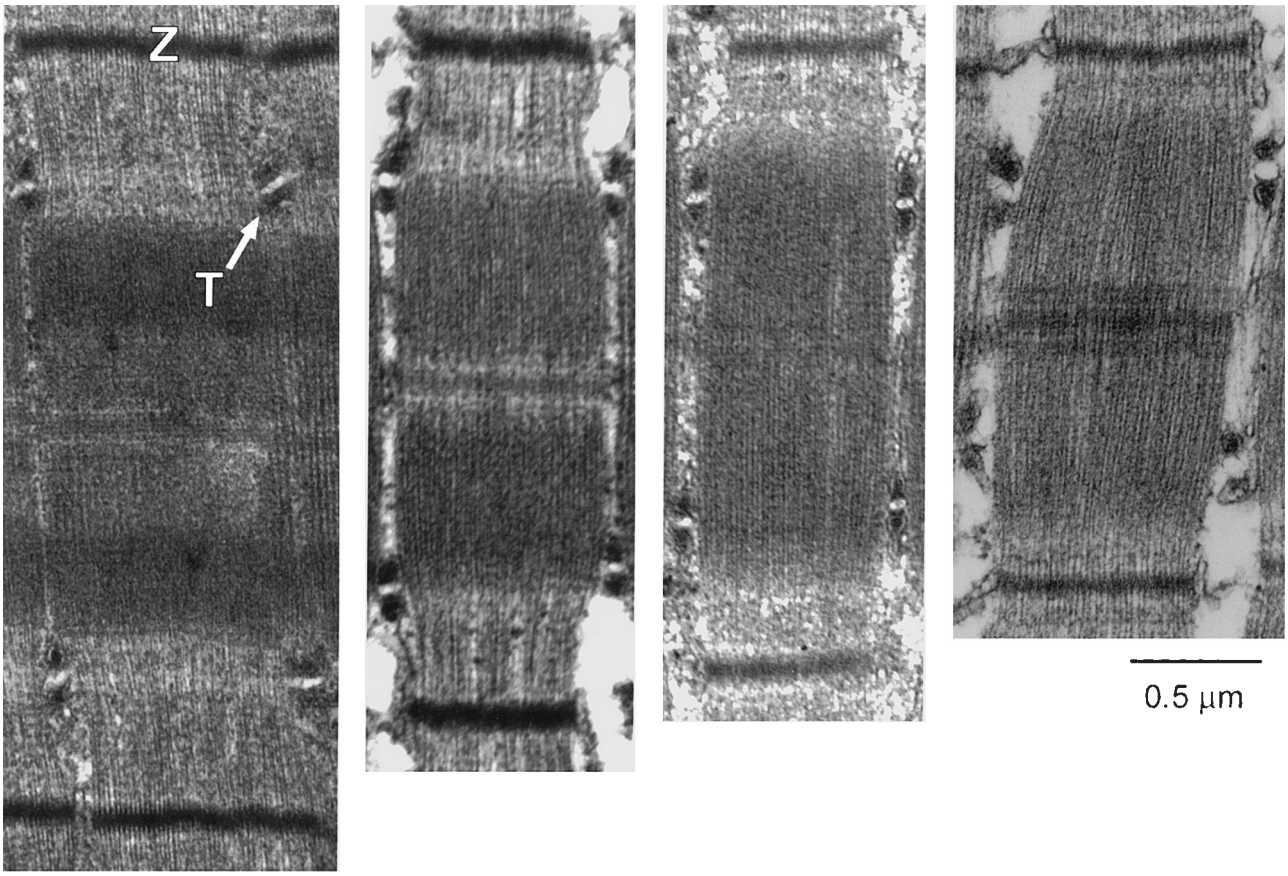

Fig. 2. Electron micrographs of longitudinal sections from four sarcomeres. The approximate sarcomere lengths from right to left are 2.1 μm (CDF42), 2.4 μm (CDF42), 2.6 μm (CDF43) and 3.0 μm (CDF43). All sarcomeres are aligned along one Z-band. The distance between the Z-band (Z) and triads (T) is relatively constant for the different sarcomere lengths.

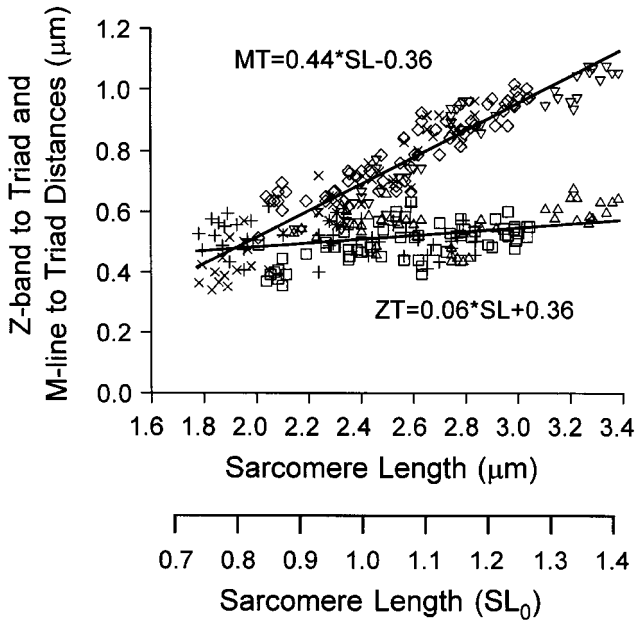

Fig. 3. Plot of Z-band to triad distance (ZT) and M-line to triad distance (MT) respectively v. sarcomere length (SL). Data are from all three animals: CDF42 (+, ×), CDF43 (□, ◇) and CDF49 (△, ▽). Best-fit lines to the data and the associated equations are included on the plot.

Our results are in qualitative agreement with those presented by Bartels and colleagues (1979) using rat semitendinosus. They observed a relatively constant ZT (their Fig. 6) for SL ranging from 2.0–3.7 μm (estimated mean ZT for all SL ~0.6 μm). Our results and those of Bartels and colleagues are in disagreement with those presented recently by Takekura and colleagues (1996) using chemically skinned rat EDL fibres. They observed an initial slope between ZT and SL of 0.25, four times greater than our observed slope of 0.06. We suggest that there are three possible explanations for the discrepancy between our slope of 0.06 and their slope of 0.25. Takekura and colleagues used chemically skinned fibres in their study, while we used a whole-muscle preparation. It is possible that the chemical skinning or dissection procedure used by Takekura and colleagues could have adversely affected the SR in some as-yet-unidentified manner. Takekura and colleagues examined a much greater range of sarcomere lengths than we did, from 1.0–5.0 μm compared with our range of 1.8–3.4 μm. Their data show a fairly consistent slope over that entire range, but stretching to the extreme lengths may have overstretched and changed the mechanical properties of various structures within the muscle.

Lastly, the possibility exists that there is a difference between cat and rat skeletal muscle, or more specifically between cat CF and rat EDL. Neither feline CF nor rat EDL show any distinguishing characteristics to suggest they are unique among hindlimb muscles, except that they are both composed homogeneously of fast-twitch fibres (Ariano *et al*., 1973; Brown *et al*., 1998). The data of Takekura and coworkers from rat EDL are also different from those of Bartels and colleagues from rat semitendinosus, which argues against a species-related phenomenon. We thus tend to favour procedural artefacts as the most likely cause of the differences between studies.

Structural implications

Our findings appear to agree with the known morphological structure of the SR. Current evidence suggests that the SR is attached to the main structural elements of the sarcomere with short, radially-oriented intermediate filaments only at the level of the Z-band (Walker *et al*., 1969; Nunzi & Franzini-Armstrong, 1980; Flucher *et al*., 1993). No other physical connections between the SR and any other structural elements in the sarcomere have ever been reported. This morphology suggests that the only structure supporting triad location is the SR itself. Interestingly, the SR is thought to be distributed in a non-homogeneous fashion on the surface of the sarcomere, appearing as a single sheet on the M-line side of the triad and as a three-dimensional multilayered sheet on the Z-band side of the triad (Ogata & Yamasaki, 1985). It is possible that this distribution results in a stiffer SR on the Z-band side of the triad than on the M-line side, providing a simple mechanism to explain our findings. However, inferring mechanical strength from morphology is speculative, and direct measurements of SR stiffness would be required to test our hypothesis.

Functional implications

At some point during mammalian evolution, triads moved away from the Z-line (where they are located in amphibians: Smith, 1966; Eisenberg, 1983; Franzini-Armstrong, 1994) to the mid-region of the thin filaments. One plausible reason for this change (which assumes that it was advantageous) would be to reduce the mean diffusion distance for calcium released from the terminal cisternae, thereby increasing the rate of rise of cross-bridge formation. Although there is no direct experimental evidence to support this hypothesis, modelling work by Cannell & Allen (1984) has suggested that diffusion distances may indeed be important. This hypothesis is consistent with the observation that triads have also moved away from the Z-line in specialized structures with very fast contraction times (e.g. toadfish swimbladder: Smith, 1966; Rome *et al*., 1996). Future studies may be able to clarify the functional implication of triad movement through direct observations of calcium diffusion within a single fibre.

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References

ARIANO, M. A., ARMSTRONG, R. B. & EDGERTON, V. R. (1973) Hindlimb muscle fiber populations of five mammals. *J. Histochem. Cytochem*. **21**, 51–5.

BARTELS, E. M., SKYDSGAARD, J. M. & STEN-KNUDSEN, O. (1979) The time course of the latency relaxation as a function of the sarcomere length in frog and mammalian muscle. *Acta Physiol. Scand*. **106**, 129–37.

BROWN, I. E., LIINAMAA, T. L. & LOEB, G. E. (1996) Relationships between range of motion, L_0 and passive force in five strap-like muscles of the feline hindlimb. *J. Morphol*. **230**, 69–77.

BROWN, I. E. & LOEB, G. E. (1998) Post-activation potentiation – a clue for simplifying models of muscle dynamics. *Am. Zool*., in press.

BROWN, I. E., SATODA, T., RICHMOND, F. J. R. & LOEB, G. E. (1998) Feline caudofemoralis muscle: muscle fibre properties, architecture, and motor innervation. *Exp. Brain Res*., in press.

CANNELL, M. B. & ALLEN, D. G. (1984) Model of calcium movements during activation in the sarcomere of frog skeletal muscle. *Biophys. J*. **45**, 913–25.

CUTTS, A. (1988) The range of sarcomere lengths in the muscles of the human lower limb. *J. Anat*. **160**, 79–88.

DIMERY, N. J. (1985) Muscle and sarcomere lengths in the hindlimb of the rabbit (*Oryctolagus cuniculus*) during a galloping stride. *J. Zool*. **205**, 373–83.

EISENBERG, B. R. (1983) Quantitative ultrastructure of mammalian skeletal muscle. In *Handbook of Physiology: Skeletal Muscle* (edited by PEACHEY, L. D. & ADRIAN, R. H.), pp. 73–112. Baltimore: Williams and Wilkins.

FLUCHER, B. E., TAKEKURA, H. & FRANZINI-ARMSTRONG, C. (1993) Development of the excitation–contraction coupling apparatus in skeletal muscle: association of sarcoplasmic reticulum and transverse tubules with myofibrils. *Dev. Biol*. **160**, 135–47.

FRANZINI-ARMSTRONG, C. (1994) The sarcoplasmic reticulum and the transverse tubules. In *Myology* (*2nd edn*) (edited by ENGEL, A. G. & FRANZINI-ARMSTRONG, C.), pp. 176–99. New York: McGraw-Hill Inc.

HERZOG, W., KAMAL, S. & CLARKE, H. D. (1992) Myofilament lengths of cat skeletal muscle: theoretical considerations and functional implications. *J. Biomech*. **25**, 945–8.

NUNZI, M. G. & FRANZINI-ARMSTRONG, C. (1980) Trabecular network in adult skeletal muscle. *J. Ultrastruct. Res*. **73**, 21–6.

OGATA, T. & YAMASAKI, Y. (1985) Scanning electron-microscopic studies on the three-dimensional structure of sarcoplasmic reticulum in the mammalian red, white and intermediate muscle fibers. *Cell Tissue Res*. **242**, 461–7.

ROME, L. C., SYME, D. A., HOLLINGWORTH, S., LINDSTEDT, S. L. & BAYLOR, S. M. (1996) The whistle and the rattle: the design of sound producing muscles. *Proc. Natl Acad. Sci. USA* **93**, 8095–8100.

SMITH, D. S. (1966) The organization and function of the sarcoplasmic reticulum and T system of muscle cells. *Prog. Biophys. Mol. Biol*. **16**, 109–42.

TAKEKURA, H., KASUGA, N. & YOSHIOKA, T. (1996) Influences of sarcomere length and selective elimination of myosin filaments on the localization and orientation of triads in rat muscle fibres. *J. Muscle Res. Cell Motil*. **17**, 235–42.

WALKER, S. M., SCHRODT, G. R. & BINGHAM, M. (1969) Evidence for connections of the sarcoplasmic reticulum with the sarcolemma and with the Z-line in skeletal muscle fibers of fetal and newborn rats. *Am. J. Physiol*. **48**, 63–77.