Changes in human skeletal muscle induced
by long-term eccentric exercise

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Summary. The fine structure of muscle fibres from *m. vastus lateralis* of nine healthy males (mean age 26 years) was investigated. Four individuals constituted non-exercised controls while five subjects participated in a two-months eccentric muscular training program. Specimens from the controls showed a well-preserved, regular myofibrillar band pattern while changes in the myofibrillar architecture were constantly found in specimens taken after the training program. These changes consisted of Z-band alterations, Z-bands being out of register, extra sarcomeres, Z-band extensions and bisected Z-bands. Between the separated Z-band halves, thin and thick myofilaments as well as abundant glycogen particles and/or ribosomes, were observed. Type-2 (fast-twitch) fibres were predominantly affected. Contrary to the controls the trained individuals constantly showed a greater variation in sarcomere lengths in Type-2 fibres than in Type-1 fibres.

It is concluded that muscular work of high tension can induce fine-structural alterations. When repeated over a long period of time, extreme tension demands seem to initiate reorganization in the muscle fibres, predominantly in the, ultrastructurally defined, Type-2 fibres. This adaptation probably results in a better stretchability of the muscle fibres, reduces the risk for mechanical damage and brings about an optimal overlap between actin and myosin filaments.

Key words: Skeletal muscles - Myofibrils - Ultrastructure - Exertion - Man

Tension is an important factor influencing the longitudinal growth of skeletal muscle fibres (Williams and Goldspink 1971; Tabary et al. 1972; Barnett et al. 1980; Holly et al. 1980). Both the length of individual sarcomeres and the number of sarcomeres are influenced (Tabary et al. 1972; Tardieu et al. 1977). Elongation of sarcomeres is probably only a transient effect (Williams and Goldspink 1978; Barnett et al. 1980), whilst the increase in the number of sarcomeres appears to be a long term change (Williams and Goldspink 1971; Tabary et al. 1972). Thus far experiments have used animals. Several of these have required denervation of muscles so that they could be stretched by the action of their antagonists (Gutman et al. 1971; Goldberg et al. 1975). Other models have employed cast-immobilization of muscle in a lengthened position (Tabary et al. 1972; Williams and Goldspink 1973; Williams and Goldspink 1978; Spector et al. 1982) or synergistic tenotomy (Schiffino and Hanzlikova 1970; Goldberg et al. 1975; Hofmann 1980). However, results of passive stretch induced growth of non-immobilized, non-denervated chicken wing muscles have been presented (Holly et al. 1980; Barnett et al. 1980). In addition, passive stretch appears to have a different effect on twitch and tonic muscles (Holly et al. 1980).

By employing autoradiography and other techniques, some workers have found that the new sarcomeres are formed at the end of the muscle fibre (e.g. Williams and Goldspink 1971). However, there are reports showing sarcomere generation taking place within the muscle fibre itself (Schmalbruch 1968).

The mechanism and the anatomical location of the length growth process is still obscure although several studies indicate that the Z-disc is the origin of the sarcomere-genesis (Kelly 1968; Bishop and Cole 1969; Legato 1970; Jakubiec-Puka et al. 1982). If the Z-disc does serve as origin for formation of new sarcomeres and if the crucial stimulus for protein synthesis under physiological conditions is the tension put on the fibres, one might expect sustained stretching during simultaneous contraction to induce growth, possibly by lengthening of the muscle fibres. In parallel to this report morphological and strength performance data after eccentric training have been evaluated (Fridén et al. 1983b). However, it soon became obvious that several fine structural alterations occurred in the muscles. Therefore, in this report results of a more detailed analysis of these findings are described and discussed.

Materials and methods

Subjects. Muscle biopsy was obtained from 9 healthy male physical education students (mean age 26 years, range 21 to 31 years). Five of these participated in an eccentric muscle training program involving the thigh muscles (see below). The remaining four individuals constituted the control group.
All individuals were informed about the significance of the experiment and gave consent. The study was approved by the Ethical Committee of Umeå University.

**Training.** Exercise was performed on a bicycle ergometer modified for use in eccentric work (Bonde-Petersen 1969). The subjects cycled 2–3 times per week for 8 weeks at a progressively increasing, individually adjusted load (Fridén et al. 1983b). On every occasion they cycled until they became severely fatigued, i.e. 12–30 min.

**Muscle biopsy.** Under local anesthesia open surgical biopsies were taken from the distal portion of *m. vastus lateralis* of the right leg from controls and exercised individuals. The controls did not perform any regular exercise during the week prior to the biopsy while the biopsies from the exercised individuals were taken three days after the last bout of eccentric exercise. Care was taken not to damage the muscle fibres mechanically throughout the procedure. Bundles of fibres, 8 to 10 mm in length and 4 to 5 mm in diameter, were excised together with the fascia.

**Preparation for electron microscopy.** The biopsy specimen was mounted at its approximate rest length with pins on a cork plate. Fixation was carried out in ice chilled 2.5 per cent glutaraldehyde in an isotonic Tyrode’s buffer solution overnight. While suspended in the buffer the mechanically undamaged middle portion of the biopsy was transversely cut into slices about 5 mm in length. From one of these, eight to ten tissue pieces were post-fixed for 2 h in one per cent osmiumtetroxide, dehydrated in graded series of acetone and infiltrated with Vestopal. Two to four tissue blocks per specimen were chosen at random. Each of these was semithin sectioned (1 μm) for light microscopy and the sections were stained with toluidine blue. A selected area containing 15–25 mechanically undamaged and longitudinally oriented muscle fibres was trimmed and ultrathin sections (60 nm) were cut for electron microscopy. The sections were contrasted with uranyl acetate and lead citrate. Further details including sampling procedure are given elsewhere (Sjöström et al. 1982b).

**Determination of the relative frequency of Z-band streaming.** Randomly taken micrographs (30–40 per individual, original magnification ×4400) were used to determine the occurrence of Z-band changes. The areas to be photographed were chosen by the raster pattern scanning rules (Weibel and Bolender 1973). Alignment between the muscle fibre axis and the copper bars resulted sometimes in “random” selection of several areas from the same fibre. In such cases only the first area was photographed. Each micrograph with one or many areas of extended Z-band material (extending over at least one fourth of the I-band width) was counted. Micrographs with Z-band extensions involving at least an entire sarcomere were considered separately.

**Classification of fibres.** The fibres were classified into Type 1 and Type 2 according to criteria defined elsewhere (Sjöström et al. 1982a). Thus, fibres with M-bands showing all five M-bridges with equal density, were classified as Type 1 fibres. All other fibres were termed Type 2.

**Measurement of sarcomere length.** Micrographs of low magnification (final magnification ×3000) were used for this purpose. The number of sarcomeres along a 50 micrometer stretch of fibre was determined. Measurements were made at three different points on each fibre, avoiding areas located near the sarcolemma (<10 micrometer). Ten fibres, from the same block, of Type 1 and Type 2, respectively, were analysed per individual. The coefficient of variation of sarcomere lengths for each individual and fibre type was calculated.

**Statistical methods.** The mean sarcomere lengths of fibres from untrained and trained muscles were compared by Mann-Whitney’s non-parametric test.

**Results**

**Control subjects.** Longitudinal survey sections showed a regular myofibrillar band pattern. However, Z-band streaming sometimes occurred, i.e. in 6 out of 162 (4%) randomly obtained micrographs. One per cent of the micrographs contained one or more regions with extensions of Z-band material involving at least an entire sarcomere (cf Fridén et al. 1983b). Difference in the incidence of Z streaming between Type 1 and Type 2 fibres could not be reliably determined in the control material. Average sarcomere length was 2.92 μm in Type 1 and 2.89 μm (n.s.) in Type 2 fibres, respectively. The range of sarcomere lengths in both fibre types is shown in Table 1.

**Trained subjects.** Longitudinal survey sections frequently showed myofibrillar disturbances (mainly involving the Z-bands) (Fig. 1). Fibres with anomalous Z-band configurations were found in 42 out of 152 (28%) of the randomly obtained micrographs. However, only 5 per cent of the micrographs contained myofibrils with at least one area of Z-band material extending over a whole sarcomere (cf Fridén et al. 1983b). The remaining Z-band anomalies occurred in essentially two different forms: either as Z-band widening and Z-band density extensions over parts of the sarcomere (Figs. 2–4) or bisected Z-band (Fig. 5a–b). All these changes occurred both just beneath the sarcolemma and deep in the fibres. Between the separated Z-band halves, both thin and thick filaments could be detected al-