EXERCISE-INDUCED MUSCLE SORENESS

A qualitative and quantitative study of human muscle morphology and function

by

Jan Fridén

Umeå 1983
ABSTRACT

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Jan Fridén, Department of Anatomy, University of Umeå, Sweden.

Exercise-induced muscle soreness is characterized by stiffness, tenderness and pain during active movements and weakness of the affected musculature the days after unusually or particularly heavy work. The most pronounced subjective symptoms do not arise immediately but rather between a couple of hours to some days after the exercise (a delayed-onset of muscle soreness), the intensity of pain is greatest about 48 hours after the work. A particular association exists between muscle soreness and eccentric contractions. Despite the fact that muscle soreness is a well known phenomenon in the sphere of sports as well as working life, the pathophysiological mechanisms underlying this are still not understood.

In the present study a detailed analysis of human muscle fibre population structure after high tension work (eccentric exercise) that gave rise to muscle soreness, was carried out. The objective was to elucidate how fibres of different types are influenced by repeated muscle contractions reaching extreme tension levels using qualitative and quantitative light and electron microscopic techniques. It was hoped that such morphological analysis would provide a basis for discussion of possible causes for muscle soreness. The muscle function after the work was measured by isokinetic methods.

To improve the basis for the ultrastructural analysis the fibre populations in untrained and endurance trained human m. vastus lateralis of age-matched individuals were classified into different fibre type groups according to their ultrastructure. The selective glycogen depletion from Type 1 fibres seen after long term submaximal work, visualized electron microscopically with PA-TSC-SP staining, substantiated the usefulness of the appearance of the M-band to differentiate between fibre types. Stereological data showed that neither volume density of mitochondria nor of lipid droplets provide sufficient criteria to differentiate between fibre types.

After an eccentric exercise regimen sore muscles (m. soleus or m. vastus lateralis) showed disturbances of the cross striated band pattern. Fibres with disorganized myofibrillar material made up 1/3, 1/2 and 1/10 of the analysed material, 1 hour, 3 and 6 days after exercise, respectively. The myofibrillar lesions were preferably localized in the Z-band. This showed streaming, broadening and sometimes total disruption. The Type 2 fibres were most affected.

The reduction of strength was greatest with the most rapid contractions. Strength remained decreased the period when the structural damage was most pronounced. Eight weeks of eccentric muscle training reduced all the above negative effects.

The results indicate that the Z-disc constitute the weak link in the myofibrillar contractile chain at high muscle tensions. It is suggested that the myofibrillar lesions are a direct result of mechanical tearing. Rupture of myofibrils is thought to result in formation of protein components and a consequential release of protein bound ions that via osmosis result in oedema and soreness. Training, using eccentric contractions over a long period of time leads to adaptations at the fibre level by a reorganization of the contractile apparatus as well as an optimization of nervous coordination.

Key words: Human, exercise, exertion, eccentric contractions, muscles, muscle strength, muscle soreness, fibre types, myofibrils, muscle proteins, histocytochemistry, ultrastructure, stereology.

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"It must be considered somewhat unreasonable to postulate structural damage to a tissue, caused by the very function for which it is specifically differentiated," deVries (1966)
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JAN FRIDÉN

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<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>CK</td>
<td>creatine kinase</td>
</tr>
<tr>
<td>EM</td>
<td>electron microscope</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyograph</td>
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<tr>
<td>IEMG</td>
<td>integrated electromyograph</td>
</tr>
<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
</tr>
<tr>
<td>LM</td>
<td>light microscope</td>
</tr>
<tr>
<td>mATPase</td>
<td>myofibrillar adenosine triphosphatase</td>
</tr>
<tr>
<td>μm</td>
<td>micrometer, micron</td>
</tr>
<tr>
<td>MVC</td>
<td>maximal voluntary contraction</td>
</tr>
<tr>
<td>NADH-TR</td>
<td>nicotinamide-adenine dinucleotide-tetrazolium reductase</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>Nm</td>
<td>Newton meter</td>
</tr>
<tr>
<td>PAS</td>
<td>periodic acid Schiff reaction</td>
</tr>
<tr>
<td>PA-TSC-SP</td>
<td>periodic acid-thiosemicarbazide-silver-proteinate</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
</tr>
<tr>
<td>TPNH</td>
<td>triphosphopyridine nucleotide</td>
</tr>
<tr>
<td>V_{ii}</td>
<td>volume density of lipid droplets</td>
</tr>
<tr>
<td>V_{mit}</td>
<td>volume density of mitochondria</td>
</tr>
<tr>
<td>VO_{2-max}</td>
<td>maximal oxygen uptake, liters • min^{-1}</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cells</td>
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REPORTS ON WHICH THIS THESIS IS BASED

This thesis is based upon the following publications and manuscript, reference to which will be made by citation of the appropriate Roman numerals:


II. Fridén J., Sjöström M.: Muscle fibre type characteristics of endurance trained and untrained individuals. (*Submitted for publication.*)


BACKGROUND TO THE PRESENT INVESTIGATION

INTRODUCTION

The incidence of localized or diffuse muscle soreness as an aftermath of unusually strenuous work is a problem found in both sports medicine and occupational and environmental health. It is well-known that individuals who have been inactive for an extended period of time discover that when they engage in strenuous physical activity, pain is often felt in the affected muscles during activity, as well as stiffness and soreness occurring several hours or days later. The objective signs are firm, tender and weak musculature. According to Talag (114) the soreness may be classified as either temporary or residual (delayed).

TEMPORARY MUSCLE SORENESS

Temporary soreness occurs during the terminal stages of fatiguing exercise. It may persist for a couple of hours and presents no lasting problems. It is accompanied by stiffness and decreased strength but the pain is generally moderate and only aggravated slightly by active movements. The primary causes are thought to be the biochemical end-products of metabolism affecting free nerve-endings and local tissue oedema (8, 17, 48, 54). Intense exercise accelerates glycolysis which leads to increased lactic acid production and intracellular acidosis (for review, see 50). The decreased pH results in an inhibition of the Ca^{2+}-activated actin-myosin interaction and reduction of mechanical tension.

RESIDUAL (DELAYED) MUSCLE SORENESS

The symptoms start a couple of hours to a day after exercise and last for several days. The site of tenderness has not been definitively localized although some studies indicate that the pains are generally experienced in those parts of the muscle where connective tissue is most abundant, i.e. at the myotendinous junction (8, 67, 68, 84).

EXERCISE CAUSING MUSCLE SORENESS

Although all forms of work, to which the individual is unaccustomed, can give rise to muscle soreness, post-exercise muscle discomfort and stiffness is primarily associated with work involving high muscle tension. A particular association between muscle soreness and eccentric contractions (negative work) has been described (e.g. 8). Going down stairs involves eccentric work. However, even though one goes down stairs just as much as up stairs, there are surprisingly few studies on peripheral effects of eccentric work. Eccentric contractions are characterized by elongation of the muscle at the same time as contraction. With a given submaximal contraction power, an eccentrically contracted muscle uses less oxygen (1, 2, 7, 72, 73) and ATP (56) than a corresponding concentric contraction, also fewer motor units are activated for any given load in eccentric work (14).

TECHNIQUES USED TO STUDY MUSCLE SORENESS

Human muscle sore from exercise, has been investigated using several techniques:
- Ergographic techniques
- Biochemical techniques
- EMG techniques
- Morphological techniques
- Rating scale techniques

When studying muscle soreness the investigator is dependent upon the subjects ability to describe the discomfort experienced. Therefore, in the following text, results from animal experiments will only be referred to exceptionally or when the results are of principal importance.

Ergographic techniques. At the beginning of this century it was found, by means of ergographic techniques that untrained individuals suffered from muscle soreness after heavy resistance work (54). The soreness was accompanied by loss of contraction strength.
It was concluded that soreness had its origin in "some sort of rupture within the muscle itself" or in the connective tissue which transmits the pull of the fibre to the tendon. The loss of contractile strength was assumed to be due to a reduction in the functional cross section of the muscle. A similar conclusion was reached by Hettinger (51) who considered the soreness experienced after intense conditioning to be due to rupture of muscle fibres and/or sarcolemma. The decrease in measured strength found by both these authors is in agreement with the results of Talag (114) who also found that muscular strength remained depressed throughout the period of soreness. Talag suggested that the pain caused inhibition of effort. This would contribute to the inability of muscles to exert their full force. No suggestions for the exact mechanism underlying the symptoms were presented.

**Biochemical techniques.** A disease of muscle called *myopathia e functione*, which seems to be identical with delayed muscle soreness, was described by Helweg (48). He expressed the belief that the swelling of the muscle was due to "physical or chemical" alterations within the muscle and soreness being a result of local accumulation of lactic acid, though this conclusion was not reinforced by experimental results. Because of lack of evidence, Hill (52) dismissed the assumption that stiffness was due to unusual acidity. Nevertheless, it is still widely believed that lactic acidosis is a causative factor of muscular soreness (4).

An increase in water and chloride content and an increase in the weight of exercised muscles in rabbits 24 hours after heavy work was found by Brendstrup (17). The oedema further increased the following day and disappeared after six days. Brendstrup stated that the time course for the development of sore muscles and the occurrence of oedema coincided. Therefore, he concluded that the sensation of pain was due to the oedema. An increase in soreness in actively contracting muscles being explained by the further tension developed in fibrils of the intramuscular connective tissue. Increased limb volume 24, 48 and 72 hours after exercise with subsequent soreness was reported by Talag (114).

Abraham (3) found myoglobin in the urine in 88 per cent of subjects who suffered from exercise-induced muscle soreness. However, in a second study 92 per cent of the subjects who performed exercise without experiencing subsequent soreness had myoglobinuria. It was suggested that myoglobin release is not specifically correlated with the development of soreness but rather appears with normal exercise. On the other hand, Abraham was able to demonstrate an increased ratio of hydroxyproline/creatinine in urine collected over the whole day of maximal soreness. Since hydroxyproline has been shown to be a specific breakdown product of connective tissue (65) Abraham concluded that exercise-induced soreness could be related to disruption of the connective tissue elements in the muscle and/or their attachments.

Recently, Watrous and coworkers (126) were able to demonstrate that delayed muscle soreness after downhill running was not reflected by significant elevations in lactate. In a similar experiment Schwane and coworkers (107) investigated the plasma CK, LDH, LDH isoenzyme activities and WBC content in sore muscles. CK was elevated 1 and 2 days after exercise while LDH and WBC counts were unchanged. It was concluded that plasma CK levels could reflect events associated with soreness and that the experiment did not cause an increase in circulating WBC. Frequent reports of elevated serum CK level after exercise with and without subsequent soreness have been presented (e.g. 44, 81, 109). The elevation of CK seen has been interpreted to be due to a leakage of this enzyme through cell walls. However, the changes in enzyme activity levels are non-specific, i.e. muscle fibres in sore muscles are not necessarily more permeable than muscles from which no pains are experienced after exercise.

Animal experiments have shown that sublethal and lethal fibre injuries and an inflammatory response occur (98, 99, 122-124) after high load or endurance exercise. A striking feature is the increased activity of acid hydrolytic enzymes after exhaustive exercise probably indicating an accelerated intracellular protein degradation (10). Armstrong and coworkers (5) found a great elevation (248 per cent) in the activity of glucose-6-phosphatase in rat *m. triceps brachii* 48 hours after downhill running. In a recent study Armstrong and others (6) found that plasma CK and LDH were still elevated 2 days after exercise though only in the rats which had been subjected to eccentric load. They concluded that exercise-induced muscle inflammation is primarily due to the negative component of dynamic exercise.

**Electromyographic techniques.** Asmussen (8) confirmed the findings of Abbott and coworkers (1, 2) in that, during eccentric contractions as compared to concentric contractions, fewer motor units are needed to produce the same tension. Based on these findings Asmussen assumed that the tension per active unit, would consequently be greater in negative work and
the risk of damage to the muscle and hence soreness would increase. The effect of eccentric and concentric muscle conditioning on muscle tension and IEMG was studied by Komi and Buskirk (67). In the early stages of conditioning the subjects in the eccentrically exercised group experienced soreness and a concomitant drop in maximum strength. In a later study, Komi and Viitasalo (70) found an increased neural activation for a given tension in sore muscles. The delayed recovery of strength after eccentric fatigue was suggested to be due to changes in the muscles “other than lowered ATP levels”. Real differences between sore and normal muscles have also been described by DeVries (28, 29). This statement is based upon the observation that surface EMG indicated a greater resting activity in sore muscles than in controls. DeVries proposed that the soreness was caused by tonic spasms in localized motor units and the severity of pain was considered to be directly related to the number of motor units involved. DeVries’ spasm theory to explain localized soreness implied that exercise above a minimal level caused ischaemia and pain, which brings about a protective, reflex, tonic muscle contraction. The tonic contraction results in a localized ischaemia of the muscle and leads to a vicious cycle. Furthermore, DeVries postulated that soreness could be prevented by periodic stretching of the affected muscle (28). DeVries results have not been confirmed. Davies and White (27) measured tetanic and twitch tension after eccentric and concentric contractions in m. triceps surae. They suggested that prolonged negative work, during which the muscle was repeatedly stretched during its contracted phase brings about soreness and weakness. Moreover, Davies and White found that muscles were weaker but not more fatiguable following negative exercise. They concluded that the contractile protein machinery was directly damaged by the repeated stretching of the muscles.

Pain and fatigue was studied by Newham and co-workers (84) after concentric and eccentric muscle contractions in m. quadriceps. Pain and tenderness developed solely in the muscle which had contracted eccentrically. The total electrical activity did not alter significantly during delayed onset soreness, thus no evidence for changes in motor unit recruitment patterns which would result in fatigue or inhibition of contraction of painful areas was found. The authors suggested that mechanical stress and trauma could explain the measured reduction in maximal voluntary force, the increase in electrical activation for a given muscle tension (cf 70) and the extreme low-frequency fatigue.

Before the previous studies on the morphological response of the muscle fibre population to unusually high functional demands can be considered the normal muscle fibre structure and the properties of different fibre types must be discussed. Structure of normal muscle fibre (fig 1). Striated skeletal muscles are composed of multinuclear cylindrical fibres, 10-100 microns in diameter and often several centimeters long. The entire fibre is surrounded by a basement membrane which when combined with the fibre plasma membrane was historically termed the sarcolemma (12, 16). The bulk of the muscle fibre consists of myofibrils which result from a repetition of sarcomeres and are packed in parallel with the long axis of the fibre. The sarcomeres are limited by the Z-discs and composed of arrays of thin (7 nm) actin-containing and thick (12 nm) myosin-containing myofilaments giving rise to the I- and A-bands. In the middle of the A-band there is a lighter zone, the H-zone. In the middle of the H-zone the M-band is observed. At rest the sarcomere length is about 2.3 μm and decreases during muscle contraction. The A-band has a fixed length of 1.57 μm, whereas the I-band varies in length according to degree of contraction. Normally the Z-bands, as well as I- and A-bands of adjacent myofibrils lie in register across the fibre. The Z-discs of adjacent myofibrils are not continuous with each other but are interconnected by 10-nm filaments (intermediate filaments, skeleton, desmin) (for review, see 79).

Other components of the sarcoplasm are nuclei, Golgi apparatus, mitochondria, glycogen granules, lipid droplets, ribosomes, polysomes, transverse tubular or T-system and the SR. The nuclei lie immediately under the plasma membrane with their long axis parallel to that of the fibre. Mitochondria occur both between myofibrils and close to the plasma membrane. The volume fraction of mitochondria correlates well with the actual endurance capacity of the muscle fibre (53, 130).

Glycogen granules are found under the plasma membrane, around the mitochondria and between myofibrils and myofilaments (fig 11a, b). Lipid droplets are found under the plasma membrane and near the mitochondria on either side of the Z-band.

The SR is a system of parallel tubules running between the myofibrils which preferentially sequesters calcium. At the junction of the A- and
I-bands the lateral ends of the sarcoplasmic tubules are distended into lateral sacs. These sacs make connections termed triads with the transverse tubular system (T-system) which lies around the myofibrils at the junction of the A- and I-band. The triads are specialized structures and are concerned with the excitation and contraction of the muscle fibre (55).

Enzyme histochemical characteristics and physiological properties of the muscle fibres. The most frequently used morphological method to distinguish between fibres with different properties is based on staining for LM of thin sections from frozen but chemically untreated muscle biopsies. Staining for mATPase is the most commonly used staining procedure in both experimental and clinical work. This procedure enables discrimination of two or three main groups of fibres in all mammalian species. Evidence has been presented that there is a correlation of histochemical and physiological properties, within a given muscle (at least concerning animal muscles) (for review, see 20, 21, 74). Based on this correlation a general classification, also employed in studies of human muscle fibres, has been widely applied. The exact terminology varies between different authors but the terms used can be summarized as follows:

- Type 1, "red", slow-twitch, fatigue-resistant, oxidative
- Type 2A, "white", fast-twitch, fatigue-resistant, oxidative-glycolytic
- Type 2B, "white", fast-twitch, fatigue-sensitive, glycolytic
- Type 2C, generally regarded as an undifferentiated precursor prior to the adoption of a true fibre type or as a dedifferentiated fibre.

One must bear in mind that conclusions regarding fibre type properties based on histochemical stainings, such as mATPase, are associated with several pitfalls. Human muscle fibres differ in many respects from animal muscle fibres. Furthermore, age, sex and physical fitness are factors that may influence upon many functional properties without changing the staining intensity of mATPase. Hence the general
fibre type classification is considered to be an oversimplification.

**Morphological techniques.** Data on muscle biopsies from human exercise-induced sore muscles is sparse. Gollnick and coworkers (40) reported unchanged ultrastructure after exhaustive dynamic exercise though mitochondrial swelling may occur (41). After repeated maximum eccentric contractions Komi and coworkers (69) could not find any ultrastructural changes either of the SR or the organization of the contractile material. In biopsies from patients suffering from interstitial *myofibrositis*, an increased amount of amorphous material between the muscle fibres was found by Awad (9). He was also able to demonstrate numerous giant myofilaments, which were considered to represent faulty myofibrillar repair. Pain and swelling was suggested to be due to an inflammation caused by muscle trauma. However, the patients did not present any history of unusual exercise.

Hecht and others (47) found fibre necrosis after endurance running in rats. van Linge (121) was able to demonstrate both degenerative and regenerative changes in muscle from rats subjected to strenuous exercise. Vihko and his coworkers have reported lethal and sublethal injuries after exhaustive exercise in rat in several studies (98, 99, 122—124). These workers assumed that the observed increase in lysosomal activation was an autophagic response probably reflecting degeneration of surviving fibres. Armstrong and coworkers (6) found, when comparing uphill, level and downhill running in rats, that injuries of the myofibrillar band pattern occurred immediately after eccentric exercise. Necrotic fibres, macrophages and satellite cells were observed 24 hours after eccentric work. The Type 1 fibres were predominantly affected. It was concluded that fibre damage was strongly associated with eccentric contractions. The implication of these results on the interpretation of delayed muscle soreness in man is still obscure.

**Rating scale techniques.** While studying exercised individuals (8) symptoms of soreness were only found in those subjects who followed a regimen of eccentric exercise. Soreness was given an arbitrary subjective classification and the results showed that the most severe pain occurred on the second post-exercise day. Discomfort was mainly experienced at the muscle attachments to tendon and fascia. Talag (114) also found that delayed muscle soreness was most intense 48 hours after exercise. Newham and coworkers (84) measured the severity and distribution of muscle tenderness in *m. quadriceps* after eccentric contractions by means of a pressure probe wrapped around the thigh. Their results showed that tenderness was localized primarily at the distal, medial and lateral parts of *m. quadriceps* while the central and proximal regions were relatively spared. At peak intensity the distribution of tenderness was more diffuse. It was concluded that eccentric contractions result in uneven tension over the myotendinous junction thereby causing mechanical damage. When comparing the subjective sensations of muscular soreness following level and downhill running Schwane and coworkers (107) found that every subject reported soreness in some muscle group at 24, 48 and 72 hours after the downhill run, exclusively.
AIM OF THE INVESTIGATION

From the previous works it is apparent that, while a number of studies concerning muscle soreness have been presented no irrefutable results about the structure of sore muscles after exercise have been presented. Accordingly most theories of the primary pathophysiological mechanisms involved in muscle soreness have been hypothetical.

Many studies inferred that muscle fibre damage was the primary lesion in sore muscles. Thus, it was considered important to carry out a thorough morphological study of the muscle fibre populations in sore muscles to elucidate any structural changes concurrent with the onset of muscle soreness.

Before a true study can be made, two prerequisites must be met:

- A suitable method to induce muscle soreness in man
- An ultrastructural knowledge of the skeletal muscle fibre type characteristics in individuals matched for age, sex and physical performance.

The detailed aim of the present study was:

- to find out whether muscle soreness is associated with ischaemic fibre necrosis or fibre ruptures;
- to qualitatively and quantitatively assess the fine structure of sore muscles;
- to evaluate whether the morphological response to exercise causing soreness is different in fibres of different types;
- to elucidate how symptoms, strength and structure are correlated in post-exercise sore muscles;
- to investigate whether a specific method of training can reduce or prevent the negative after effects seen when unusually high load is put on untrained muscles.
EXPERIMENTAL PROCEDURE

Detailed descriptions of the methods used can be found in the original papers.

SUBJECTS

In all, 35 healthy males (aged 17–35 years) volunteered for the study. The experimental groups consisted of physical education students (I, II, IV, V) or medical students (III). The runners in study I and II trained by distance running 2–3 times per week. The subjects in study III to V were physically active but not taking part in athletics. Before giving their consent all subjects were informed of the procedure and purpose of the experiment and on possible detrimental effects. The subjects were free to stop participation at any time during the study. The study was approved by the Ethical Committee of Umeå University.

EXERCISES

Prolonged dynamic exercise (I, II). The subjects took part in the Lidingö race (a long distance cross-country run of 30 km in Stockholm). The race was run on good paths in a varied terrain which included several small hills. The subjects average race time was 2 hrs 19 min (range 1 h 56'–2 hrs 42').

Single bouts of eccentric exercise (III, IV). In a pilot study subjects performed a moderate eccentric exercise (running downstairs), which primarily involves calf and thigh muscles (III). In a subsequent study individuals were subjected to an intense sustained eccentric exercise primarily involving the thigh muscles (IV). The exercise regimen employed a bicycle ergometer modified for use in eccentric work (15). The intensity of work was equivalent to concentric exercise at 80–100% of individual VO₂-max.

Prolonged eccentric training (V). The subjects were given an eccentric muscular training program using a modified bicycle ergometer (15). The subjects cycled to severe fatigue 2–3 times per week. The work intensity was gradually increased over the training period of 4 or 8 weeks.

DETERMINATION OF VO₂-MAX (IV, V)

VO₂-max was determined on a motor driven treadmill. Douglas bags were used to collect the expired air, volume measured in a Tissot spirometer and gas samples analysed with a Centronic mass spectrometer (for details, see 45). Speed and gradient on the treadmill were adjusted so that VO₂-max was achieved for each individual according to the "leveling off" criteria (31).

STRENGTH MEASUREMENTS (IV, V)

Maximal voluntary joint torques developed by the knee extensors of the left leg were measured according to the procedure described by Thorstensson and coworkers (119) using an isokinetic device (Cybex II, Lumen Inc., New York). The machine was preset to desired angular velocities (90, 180 and 300 degrees per second in study IV or 15, 60, 120 and 180 degrees per second in study V). The subjects performed two maximal contractions at each preset velocity, of which the larger was taken to be the accurate maximal force.
SPRINTING PERFORMANCE (V)

A mechanically braked ergometer was used to evaluate sprinting performance (for details, see 13). All subjects performed 20 full pedal revolutions as fast as possible and the time taken was recorded. The preset load (about 10% of body mass) was the same before and after training for each individual.

MUSCLE BIOPSY TECHNIQUE

After local skin anaesthesia (Xylocain®, 1% without adrenaline) open surgical biopsies were obtained from the right and left m. soleus (III) or the right m. vastus lateralis (I, II, IV, V). Care was taken to avoid infiltration of the anaesthetic agent into the muscle. The same surgeon took all biopsies either from a well-defined portion at the middle of the lower leg two centimeters dorsal to the medial margin of tibia (III) or 15 centimeters proximal to the lateral condyle of femur (I, II, IV, V). An incision of about three centimeters in length was made over the belly of the muscle along the fibres. The fascia was divided and a segment of muscle (8—10 mm in length and 5 mm in diameter) was carefully excised together with the fascia. Immediately after removal each biopsy was divided into two halves, one of which was prepared for histochemistry, the other for electron microscopy.

In study I, II and V control biopsies were taken from matched, non-exercised individuals. In study III and IV control biopsies were taken from subjects at least two weeks before the exercise study commenced.

MORPHOLOGICAL ANALYSES (I—V)

Preparation for enzyme histochemistry. Each sample was cut transversely into slices and oriented in OCT embedding medium (Ames Tissue-Tek) on a piece of paper and frozen in liquid nitrogen-chilled difluorodichloromethane (Freon 12) and stored at −80°C. Serial transverse sections (10 μm thick) were cut in a cryostat at −20°C and mounted on glass slides. The sections were stained with hematoxylin-eosin, treated for mATPase at pH 9.4 (88) as well as pH 4.6 and 4.2 (18, 19). Sections were also stained for NADH-tetrazolium reductase (85) or TPNH. Glycogen was visualized using the PAS reaction (90). The sections were then examined using a Leitz Dialux-20 microscope. Based on the staining properties for alkaline mATPase, at least 400 fibres from each specimen were classified into Type 1 (lightly stained) and Type 2 (darkly stained). At pH 4.6 Type 2A fibres were lightly stained and Type 2B fibres were darkly stained. The identity of the latter was checked at pH 4.2 where they only showed light staining, at pH 4.2 intermediately stained fibres were classed Type 2C.

Fibre diameter measurement. Using an ocular scale two orthogonal diameters were measured; the mean being taken as the muscle fibre diameter (106, 112). The sample size for each fibre type was 100 fibres in each biopsy. However, when less than this number of fibres of a fibre type were present all fibres of that type were measured. The measurements were always performed by the same observer to reduce interobserver variation.

Preparation for light microscopy and electron microscopy of plastic sections. The biopsy sample was pinned onto cork at its approximate resting length. The muscle was fixed overnight in ice-chilled 2.5% glutaraldehyde in an isotonic Tyrode’s buffer solution. During rinsing the middle portion of the biopsy, which was not mechanically damaged, was transversely cut into slices about one millimeter thick. One of these slices was cut into between eight to ten pieces and post-fixed for two hours in 1% osmium tetroxide, dehydrated in a graded series of acetone concentration and embedded in Vestopal. The blocks were trimmed and semithin (~1 μm) and ultrathin (~60 nm) sections were cut. The semithin sections were stained with toluidine blue and examined under a LM (Leitz Dialux-20). The ultrathin sections were contrasted with uranyl-acetate and lead citrate and examined in an EM (Philips 300 or JEOL JEM-100 CX).

Sampling and procedures for stereology (II, IV, V). Two to four Vestopal embedded blocks per biopsy specimen were chosen at random. From each of these semithin sections were cut. Longitudinal semithin sections were photographed and printed at a final magnification of 1,200 diameters (IV). Montages were made to provide an entire view of the section. Regions of myofibrillar disruption involving at least one sarcomere were encircled. The relative area of the delimited regions was determined using a point-counting procedure (fig 3) (127).

Each ultrathin section contained 15—25 mechanically undamaged and longitudinally oriented muscle fibres. One section from each block was selected and six to ten fibres were randomly chosen and selected areas of these fibres photographed. The selection criteria was that the photographed area was not located near to (within one micron) the periphery of the
Fig 3. Toluidine blue stained semithin longitudinal section of biopsy from sore muscle. A point counting procedure was used for determining the relative area of myofibrillar disintegration. Encircled examples of hits.

Fibre and therefore fibres with a profile of less than ten micron were rejected. The original magnification was 4,400 diameters and the final magnification was 13,200. One observer made all stereological measurements using a frame in the form of a double period square lattice, 150 x 220 millimeter, containing 630 test points. The frame was thrown randomly on the micrographs. $V_{mit}$ (II, IV, V) in the core of the fibres and $V_{II}$ (II) were determined.

The Z-band width was measured on the electron micrographs using an eye-piece magnifier fitted with a graticule scored at 0.1 mm intervals. Measurements were made at ten equally spaced points distributed along the Z-striation.

Fibre type discrimination and terminology at the ultrastructural level (I, II, IV, V). The fibres were classified as Type 1 or Type 2 with subdivision of Type 2 into Type 2A and Type 2B according to criteria defined elsewhere (110, 111). 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. Of these fibres, those with M-bands with the three middle M-bridges clearly visible but the two outer ones less distinct were termed Type 2A fibres. Fibres with only the three central M-bridges clearly visible were termed Type 2B. All ultrastructural fibre typing was done in a blind manner.

Ultrastructural visualization of glycogen particles (I). Ultrathin sections were cut and lifted onto Formvar coated gold grids and treated according to the PA-TSC-SP method of Thiéry (116) (modified by 117) to identify the presence of glycogen.

Lateral smearing of myofibrils (I). The axial distribution of stain when averaged across the myofibril (laterally smeared images of conventionally stained sections) were obtained by movement of the photographic paper at constant speed during printing of the micrograph on an enlarger.

STATISTICAL ANALYSES

The significance of differences between two intra-subject mean values was tested with Student’s t-distribution for dependent observations (II, IV, V) or Mann-Whitney’s non-parametric test (IV, V).

The sources of variation of $V_{mit}$, $V_{II}$ and Z-band width (dependent variables) with subject and fibre type (independent variables) were calculated by using two-way analysis of variance (II).

The ability of different ultrastructural parameters ($V_{mit}$, $V_{II}$ and Z-band width), used single or in combination, to discriminate fibres preclassified according to the M-band appearance was tested by discriminant analysis (66) (II).
RESULTS

SYMPTOMS (III—V)
All individuals who performed a single bout of eccentric exercise experienced muscular discomfort (fatigue and stiffness) during the terminal stages of activity. Furthermore, they all suffered from severe diffuse soreness in their calf (III) or thigh muscles (III—V) at least 1 to 3 though sometimes 4 or 5 days following exercise. In the training study (V) the subjects had sore muscles after each of the first 3—4 exercise bouts, i.e. during the first 1—2 weeks of training but were free from complaints following 2—3 weeks of training.

FUNCTION
In untrained individuals the concentric force exerted at all angular velocities was significantly decreased immediately after a single bout of eccentric exercise (IV). This was particularly evident at the higher speeds (180 and 300 degrees per second) and during the period of soreness (fig 4). On the other hand the subjects who had trained eccentrically (V) for 4 and 8 weeks showed no reduction in the maximal force output. In the training study it was found that, after an initial decrease in strength (during the first two weeks) all individuals improved their maximal concentric strength after 8 weeks of training (V) (fig 5).

Fig 4. Maximal exerted torque (expressed in per cent of preexercise value) of the left knee extensor muscles after eccentric exercise. *p < 0.05, **p < 0.01, ***p < 0.001.

Eccentric endurance strength was dramatically improved by training (V).
The $VO_2$-max before and after 8 weeks of training was equal (V).
Sprinting performance was slightly improved after two-months training (V).

MORPHOLOGY-LIGHT MICROSCOPY
Overall morphology (I—V). All biopsies taken before and after exercise which were investigated histochemically, showed tightly packed fibres in well organized fascicles. Neither focal nor diffuse fibre abnormalities were observed in any of the specimens analysed in paper I—IV. However, several of the biopsies taken after 8 weeks of training (V) showed small, rounded fibres as well as angulated fibres (always lightly stained at pH 9.4). Moreover, in biopsies from trained individuals the fibres showed a large variation in staining intensity. Biopsies from two individuals, taken 3 days after a single bout of eccentric exercise showed an increased number of central nuclei (IV). Central nuclei were also frequently observed in specimens obtained after 8 weeks of training (V).
Fibre type proportions (II–V) and fibre sizes (III–V). In general, the fibre type proportions remained unchanged. The only exception was a high proportion of Type 2C fibres in sections from subjects who had trained for two months (V).

The mean fibre sizes always remained unchanged. Semithin sections (III, IV). Toluidine blue stained semithin survey sections of biopsies taken after exercise showed abundant focal disturbances of the cross-striated band pattern (fig 6). The changes were observed in one third, half and one tenth of the fibres, immediately after, 3 days after and 6 days after eccentric exercise (IV). The relative fibre area occupied by the disturbances also showed a peak at the third post-exercise day.

PAS-stained frozen (I, IV, V) and plastic sections (I). PAS-stained sections from the subjects who had performed sustained, submaximal exercise (I) showed numerous glycogen depleted Type 1 fibres (fig 7). Some Type 2A fibres also showed evidence of glycogen depletion though never to the extent seen in the Type 1 fibres. Similar differences in glycogen content were confirmed in the PAS-stained longitudinal plastic sections (fig 8).

Fig 6. Toluidine blue stained survey section showing focal disturbances of the cross-striated band pattern after eccentric load.

Fig 7. Histochemically stained cross sections showing glycogen depleted Type 1 fibres after endurance running. mATPase (pH 4.2) (left), TPNH (middle) and PAS (right).

A homogeneous staining intensity was always seen immediately after a single bout of eccentric exercise (IV).

Biopsies taken immediately after maximal eccentric work from subjects who had trained for 8 weeks showed glycogen depletion in the type 2B fibres only.

MORPHOLOGY-ELECTRON MICROSCOPY

Organization of the myofibrillar material (I–V). The muscle fine structure in biopsies from both endurance runners and control individuals was normal. Myofibrillar disintegration was observed in all samples obtained after eccentric exercise (both immediately after and 3 days after), though the extent of the changes varied (fig 9).
Fig 9. Electron micrographs showing the fine structure of control biopsy (a) and of biopsies taken 3 days after eccentric exercise (b–f). x=Type 1 fibre, xx=Type 2B fibre.
The origin of the disturbances was seen to be the Z-band, appearing widened and irregular. In more extreme lesions the Z-band material extended irregularly into the A- and I-bands (fig 10a), involving one or many sarcomeres. There was also evidence for Z-band streaming over several neighbouring myofibrils. In the myofibrillar lesions the array of thick and thin filaments was obscured by deposits of dense material emanating from the Z-bands. In some sarcomeres there was an apparent loss of thick filaments. In cross-sections of affected Z-bands the normally regular filamental lattice was lost (fig 10b). Within or close to abnormal areas there was a scarcity of mitochondria and a surfeit of ribosomes.

Fig 10. Longitudinal section (a) and cross section (b) of severely disorganized Z-band material involving several myofibrils and extending into the I- and A-bands.

Fig 11. Electron micrograph of PA-TSC-SP stained longitudinal sections. The glycogen particles are located beneath the sarcolemma (a) and between the myofibrils predominantly at the level of the I-band (b). Figure c shows a Type 1 fibre of biopsy taken immediately after a long distance race.
Distribution of glycogen particles (I). Glycogen particles were abundant in all fibres from biopsies of non-exercised individuals. The particles were located in subsarcolemmal accumulations and between myofilaments, particularly at the level of the I-band close to SR and mitochondria (fig 11a, b). In biopsies taken after exercise the Type 1 fibres showed no subsarcolemmal accumulations of glycogen and intermyofilamentary particles were sparse and of smaller size (fig 11c). Trained individuals showed very few particles in the A-band of Type 1 fibres though they appeared frequently in the A-band of Type 2B fibres.

Lateral smearing of myofibrils (I). This technique allowed good resolution of differences in M-band fine structure and Z-band width between different fibre types in routinely contrasted sections.

Fibre type classification (II). Two independent observers gave the same classification for 78% of the fibres in the trained group and 71% of the fibres in the untrained individuals. Overall 217 of the 294 fibres (74%) were given the same classification by both observers. Of those fibres which were classified differently by each observer Type 1 fibres were grouped as 2A fibres and 2A fibres grouped as 2B fibres or vice versa.

Stereological data (II, IV, V). Untrained and endurance trained subjects (III). The best overall classification result was obtained when $V_{mit}$ and Z-band width were combined (66% (trained) and 63% (untrained)). Adding $V_{hi}$ did not improve the result. The best single parameter was Z-band width (60%) as less than half of the fibres would have been correctly classified by using $V_{mit}$ alone. $V_{mit}$ was higher in all fibre types in the trained group ($p < 0.001$). $V_{hi}$ was higher in Type 2A fibres only ($p < 0.01$). $V_{mit}$ was higher in Type 1 than in Type 2A and 2B fibres ($p < 0.05$ and $p < 0.025$, respectively). There was no significant difference between $V_{mit}$ in Type 2A and 2B fibres. In general, the degree of association between different morphometric parameters was weak, especially when regarding trained and untrained subjects separately.

Eccentrically exercised subjects (IV, V). $V_{mit}$ was higher in all fibre types 3 days after exercise (IV) and was slightly increased in Type 2 fibres after prolonged eccentric training (V). Z-band width was not affected by training (V). The proportion of micrographs showing Z-band streaming was 19, 20 and 1 per cent 1 hour, 3 days and 6 days after a single bout of eccentric exercise (IV). The occurrence of Z-band streaming was greatly reduced by prolonged eccentric training (V).
DISCUSSION

SYMPTOMS OF SORENESS AND ITS EFFECT ON STRENGTH

In the present study, symptoms of pronounced training-related muscle soreness and decreased voluntary strength were observed after an isolated stint of exhaustive eccentric work (cf 8, 68, 70). The rate of recovery of voluntary strength showed a good correlation with the abatement of soreness. Various authors, using different methodological approaches (particularly EMG studies), have speculated that the reduction in strength after repeated eccentric contractions may depend on damage to the contractile apparatus (27, 70, 84).

Previously it has been suggested that mechanical damage to the SR may result in less calcium being released for anyone excitatory action potential and subsequently leading to decreased tension generation (59). Another possible reason for the decrease in maximal strength and the increase in neural activity at a given muscle tension after eccentric work observed by Komi and coworkers is mechanical damage to the elastic components of muscles (68, 70). The present findings suggest that the reduction in strength may be explained by a disorganization of the contractile material within the muscle fibre. Also it is clear that pain plays a role in limiting maximal strength. It may be possible to deduce the inhibitory effects of pain by analysing MVC under local anaesthesia.

The subjects in the training study (V) showed a continuous, progressive loss of symptoms associated with muscle soreness, a minor improvement in concentric strength and a major improvement in eccentric strength in addition to a decreased preponderance for myofibrillar lesions. Training is thought to cause an optimization of neural activation of the necessary motor units (i.e. fast twitch fibres) for high tension work as well as structural adaptations at the fibre level (see below).

MORPHOLOGICAL FINDINGS AFTER ECCENTRIC LOAD

Ischaemia is either very limited or absent in muscles that become sore and as such it is not considered to have a primary causative effect. This statement is in contrast with the model for development of muscle soreness proposed by deVries, based on EMG studies (29). However, no further experimental evidence has been presented to substantiate the "ischaemia-muscle spasm" theory of deVries. No swelling of capillary endothelial cells was found. As no increase in the number of mononuclear cells was apparent, the possibility of invasive cells being a factor, inherent in the development of soreness, is also unlikely. This finding is consistent with the results of the studies on the effect of eccentric work on animals which showed that the circulating WBC count is unchanged (107). Moreover, Janssen and coworkers (57) found that an anti-inflammatory drug (flurbiprofen) had no effect on the perception of soreness after eccentric muscular activity in humans.

Here the most prominent finding was a varying degree of disruption of the contractile material, particularly the myofibrillar Z-band (fig 9, 10). However, to evaluate changes in Z-band structure the normal Z-band appearance needs to be discussed.

Ultrastructure of the Z-disc (Z-band, Z-line). In cross section the Z-disc appears as a woven basket or square lattice (fig 12). It is reported that these patterns may interchange during contraction (77). In numerous reports, involving several species, different authors have presented various models to explain the observed Z-disc ultrastructure (36, 39, 60–62, 71, 77, 80, 92, 94, 95, 120). The first structural analysis of the vertebrate Z-disc (frog semitendinosus muscle) was presented by Knappes and Carlsen (71). They proposed that each thin I-band filament from one side of the Z-disc is positioned equidistant to four thin filaments from the opposite side of the disc and inter-connected by four Z-filaments. Franzini-Armstrong and Porter (36) rejected Knappes and Carlsen’s idea of the Z-filament. Instead they suggested that the Z-disc is a membrane to which the ends of the I-filaments are attached. Reedy (92) described a woven appearance explained by four strands, unwinding on both sides the disc from each I-filament. Kelly (61) proposed that the Z-disc was formed by strands emanating from one sarcomere, interlooping with strands from the opposite sarcomere and returning to join I-filaments on the original side. Each I-filament was assumed to be split into two strands. Rowe (94)
also published a model based on looping strands. However, Rowe assumed that the hairpin-looping strands joined an adjacent I-filament on the original side, without interlinking with strands from the opposite side. Kelly and Cahill (62) proposed a model suggesting that each I-filament gives rise to four curving Z-filaments which extend to I-filaments of the opposite sarcomere. Ullrick and colleagues (120) presumed that one thin filament entering the Z-disc is in continuity with three curved Z-filaments which unite it with three other thin filaments of the same sarcomere. From this discussion it is clear that there is still considerable disagreement concerning the fine structure of the vertebrate Z-disc. Recent authors (39, 118) consider the Z-disc material to consist of terminal actin filaments arranged in a complex interdigitated fashion interlinked by oblique bridging filaments.

Much of diversity between these different models are now considered due to the different species investigated. Also the variety of Z-disc appearance has been attributed to different fixation procedures by some workers (77, 80). Landon claimed that after osmium tetroxide fixation a basket-weave pattern would be seen, while muscles fixed in glutaraldehyde and post-fixed in osmium would show Z-discs with predominantly small-square lattice. Other possible reasons for the diversity in the observed structure is the effect of variations in section thickness, section obliquity or differences due to fibre type have been discussed (see 60, 94).

**Implications of myofibrillar damage.** In a pilot study (III), it was found that pronounced Z-band disturbances were present in the first biopsy (i.e. two days after the work), though a following study (IV) confirmed that myofibrillar disruption was also present in biopsies taken immediately after excessive eccentric work. Often the normally well-organized Z-band had a ragged drawn-out appearance. Overall the ultrastructure of the sarcomere was very variable. In some regions it was normal whilst other regions showed extreme sarcomeric disruption. It has been suggested previously that Z-band streaming is predominantly subsarcolemmal and is commonly found in the vicinity of a blood vessel (76). No such pattern was apparent here and the disturbances seemed to be random both across and along the muscle fibre. The biological significance of Z-band streaming is unknown (24). However it is a common, nonspecific, finding in neuromuscular disease (for review, see 24). Fischman and colleagues (35) as well as Meltzer and his co-workers (82) have reported Z-band streaming in control subjects though these changes were less extensive than those observed in the present study. Here, the changes in Z-disc are considered to be due to mechanical rupture of the Z-disc. The extreme tension concurrent with eccentric contractions may put severe strain on the complex network existing within the Z-disc. Because of the complexity of the Z-band lattice and the diverse opinions concerning its fine structure the exact mechanism behind the development of Z-band streaming after exercise is not, at present, possible to deduce. An alternative possibility is that changes in the SR leading to calcium ion flooding, have caused a calcium-induced weakening of the Z-band (46). In the present work, however, there was no apparent sign of abnormal SR fine structure.

The structural integrity of the Z-disc as well as the maintenance of the transverse alignment of sarcomeric striations have been attributed to the existence of filamentous bridges between Z-discs and between
M-lines across the fibre axis (for review, see 78, 79). Recent structural studies of potassium-iodide extracted myofibrils have shown the existence of continuous, parallel longitudinal filaments connecting the periphery of successive Z-discs and ensheathing the entire sarcomere (125) (fig 13). The existence of this longitudinal cytoskeleton suggests that sarcomeres may be able to transfer tension even though tension generation is limited by myofibrillar damage.

**Membrane considerations.** Some studies report an increase in serum CK levels concurrent with muscle soreness after work of long duration or dynamic work involving continuous load (e.g. 44). Increases in serum CK have been suggested to be due to membrane damage (e.g. 97). Janssen and coworkers (57) were not able to detect any differences in the level of CK between exercises not giving rise to soreness (concentric) and those leading to soreness (eccentric). Therefore, the discrepancy between the results of Hagberg and coworkers and Janssen and coworkers are likely to depend on the different types of work, i.e. exercises involving continuous load (isometric work) causes more pronounced ischaemia (11) leading to increased membrane permeability (96). However, the methodology used in this study does not allow comment on the possibility of plasmalemmal damage, though no physical disruption of the membrane was seen.

The volume fraction of mitochondria was increased three days after eccentric load (IV). There are two possible explanations to this finding. It either reflects an increased number of mitochondria or an increase in mitochondrial size. There is no reason to expect the single bout of eccentric exercise to induce an increase in oxidative capacity. A more plausible interpretation is an enlargement of mitochondria due to swelling. However, great care should be taken when assessing mitochondrial swelling as the mitochondria, among electron microscopists, are known to be very sensitive to processing artefacts. Still, the increased mitochondrial volume observed in study IV may be a secondary effect of intracellular oedema caused by the release of osmotically active ions and protein components (cf discussion, paper III). The question arises whether the increased mitochondrial volume is a result of a swelling of all muscle fibre membranes. Though no stereological measurements were made on the relative SR volume a qualitative analysis of SR did not reveal any SR swelling or other damage. Disruption of the SR to explain decreased post-exercise strength as suggested by Jones (59) in
his studies of low frequency fatigue after exercise is unlikely since such damage should have caused an increased level of myofibrillar Ca\(^{2+}\). This in turn would have caused contraction clots which were not found in the present study.

**Possible role of lysosomal enzymes.** When considering studies on excessive physical work, one must also bear in mind that activation of large quantities of hydrolytic lysosomal enzymes may occur (98, 122, 123). It is unlikely that autolytic degeneration of the myofibrils is the cause of the observed changes as these occur very rapidly, within an hour of cessation of work (cf 122, 123). On the other hand, it is possible that a primary shredding of filaments, exposes contractile proteins to proteolytic attack (6). The lysosomal activation that occurs two to seven days after work (123) may account for the more extensive lesions seen in biopsies taken three days after the work as compared with those seen in biopsies taken immediately after cessation of work (IV). In the present work it was found that the myofibrillar repair process took about a week, corresponding well with the findings of Vihko and coworkers (122, 123).

**Fibre type involvement.** Morphological disturbances were found in both ultrastructurally defined (see Ultrastructural fibre type classification) Type 1 and Type 2 fibres, though were more common in the latter fibre type. This is in contrast with Vihko and his colleagues (123) and Armstrong and coworkers (6) who reported that in the rat Type 1 fibres were most susceptible to necrosis after excessive physical exercise. These authors considered the necrosis due to a greater preponderance for ischaemia or increased recruitment of Type 1 fibres. However, these authors did not employ ultrastructural methods. In a recent study Kuipers and coworkers (75) found ultrastructural signs of degeneration in both Type 1 and Type 2 fibres 24—28 hours after a single bout of submaximal exercise in rats. Also apart from possible differences in the reaction between rodent and human muscle to the work, the individual work period was much greater in the animal studies than in this study. It should be noted that the subjects in the present study could discontinue the work at any time and in some cases did so (cf IV) when the subjective discomfort became too great. This meant that the work never gave rise to true fibre necrosis.

In investigation IV no evidence for selective fibre recruitment on the basis of PAS-staining was found. Present knowledge on contraction characteristics and muscle fibre recruitment patterns during submaximal dynamic work (21, 49, 102), make it possible that both Type 1 and Type 2 fibres are involved in the development of tension during eccentric work of the type employed in this study. The intensity of work and tension demands made in investigation IV were high, and as such it is likely that even the Type 2 fibres played a considerable role in tension development. It may also be possible that the broader Z-band in Type 1 fibres (91, 110, 111) is less vulnerable to repetitive, large tension variations than the Z-bands in Type 2 fibres.

**Effect of long term eccentric training.** In the training study (V), there was no evidence of the extensive myofibrillar lesions seen after an isolated stint of eccentric work by untrained subjects (III, IV). In biopsies from the trained individuals fibre type proportions were significantly different to the control biopsies; the number of Type 2C fibres was greatly increased, probably associated with an adaptation to changed functional demands (104).

Eccentric work exposes the Z-discs to extremely powerful and repetitive tensions and as such great structural strength is needed within the disc. Three methods of myofibrillar adaptation are possible to avoid overloading and damage to the Z-disc. The first possibility is that the sarcomere length may be increased though this would result in an nonoptimal overlap between myosin and actin molecules and as such can only be a temporary solution to reduce the tension over the myofibrils (129). Another possibility is that the longitudinal sarcomere number may increase (113, 128). Since extra sarcomeres were apparent after training in the present study, it may well be that sarcomerogenesis occurs. A third possibility is that the Z-band may be strengthened by an increase in synthesised Z-band proteins (cf 30, 100) or intermediate filaments (for review, see 78, 79). Though no change in Z-band width was seen here the relatively short duration of the study does not eliminate the possibility of changes in Z-band width over longer periods of exercise. Animal studies have shown that transformation of Z-band structure takes place between one and a half and three weeks after onset of continuous electrical stimulation (30). Though conventional electron microscopy and stereology does not allow resolution of the possible cytoskeletal involvement in muscle training further studies on the influence of repetitive contractions of high tension on Z-disc fine structure and intermediate filament organization are necessary to elucidate the detailed adaptation mechanisms (see Future problems and prospects.)
Another sign of adaptation is an increase in the mitochondrial volume fraction during the first half of the training program followed by a decrease. This is interpreted as an improvement in physical coordination resulting in fewer motor units being activated. This assertion also suggests that oxygen consumption is not increased despite successive increase in load and that a displacement to an increased Type 2B recruitment (shown by PAS-staining) was a result of the training.

ULTRASTRUCTURAL FIBRE TYPE CLASSIFICATION (I, II, IV, V)

Electron microscopic studies of the reaction of muscle fibres of different types to changes in functional demands are not possible unless the fibres can be reliably distinguished from each other at the ultrastructural level. It is essential that the distinguishing criteria are not sensitive to short term changes in activity. Early studies were based on qualitative descriptions of mitochondrial content and distribution. Saltis and Mendell characterized fibre types ultrastructurally by employing diaminobenzidine staining (DAB), a presumed marker for myoglobin. Schmalbruch and Kamieniecka classified fibres by qualitative analysis of mitochondria, lipid, SR and the width of the Z-band. Other authors have presented quantitative data on fibres from individuals of different ages and different athletic capacities. However, no quantitative fibre type specific data was given by these latter authors. Since then, morphometric techniques have been widely applied to obtain criteria to distinguish fibre types. The methods have included measurements of:

- Z-band width
- Volume density of mitochondria
- Sarcotubular surface area
- Lipid content
- M-band appearance
- M-band width

However, there is still considerable controversy over ultrastructural fibre typing of human muscle fibres. This controversy is primarily dependent upon the following factors:

- Human muscle fibre types are less commonly examined at the ultrastructural level, hence workers have less experience in classifying fibre types in human muscle than in the commonly investigated lower mammals.
- The number of samples is generally small.
- The subjects are not matched for sex, age or physical performance.
- A common error is that inherent ultrastructural difference per se may exist between "the same fibre type" in different muscles and as such comparisons between different muscles are invalid.

The M-band as fibre type discriminator. In the present work M-band fine structure was used to discriminate between fibre types. Its validity was clearly shown in paper I. High standards in tissue preparation and staining procedures are necessary to ensure that the assessment of fibre type is reproducible. One must also take into account that certain differences exist between both individuals and muscles. For example in an earlier study it was found that differences between fibre types were less distinct in m. vastus lateralis than in m. tibialis anterior. The reproducibility of the use of the fine structure of the M-band as a fibre type discriminator has been found to be high despite the possible sources of error mentioned above. Using M-band based fibre typing a strong association has been shown to exist between morphological data and physical performance in subjects with intermittent claudication.

In investigation I, histochemical and electron microscopic techniques were used to define fibre types by assessing their glycogen depletion pattern. It is known that there is some selectivity of fibre type recruitment with work. Accordingly, glycogen depletion in different fibre types varies depending on the intensity and duration of the work. In study I, serial sections were treated either with conventional EM stains or with specific glycogen stains. Selective depletion of glycogen from Type 1 fibres was apparent and used in tandem with the M-band appearance to confirm Type 1 and Type 2 fibre characteristics. In study II, the correlation between results obtained by two independent observers was tested, and found to be high (about three out of four fibres were typed identically). Discrimination analysis of the results showed that they corresponded remarkably well.

A limitation of the techniques used here is that they do not give any absolute criteria for ultrastructural typing of the enzyme histochemically defined Type 2C fibres. After long term eccentric training mATPase showed that the proportion of Type 2C fibres is increased though electron microscopic analysis of these samples did not allow resolution of...
A number of interesting conclusions could be drawn regarding the fibre population. The results suggest that a fibre classification based on mitochondrial volume (cf e.g. 91) is unreliable. This is because a large overlap existed between the mitochondrial content of Type 2A and Type 2B fibres. In total, less than half of the fibres would be classified correctly on the basis of \( V_{\text{mit}} \) alone. When Z-band width alone was used as a criteria for discrimination the risk for misclassification was also considerable though better than using \( V_{\text{mit}} \). The variation in lipid content between different fibre types in both untrained and trained individuals was so large that this parameter could not be reliably used to differentiate fibre types.

The great variation in both mitochondrial and lipid volumes within the same fibre type (classified by M-band appearance) is probably the cause of the weak correlation between the different ultrastructural parameters studied here. It appears therefore that classification of muscle fibres into different types is an artificial way of looking at the fibre population and in many cases may make it more difficult to elucidate the muscle fibre dynamics. By using photometrical evaluation of the effects of sequential preincubation of mATPase Dahl (25) has shown that the fibre population represents a continuum also in respect to enzyme histochemical properties.

In summary, the results showed that M-band fine structure differs between fibre types and is useful in studying fibre type specific adaptation. On the other hand, one must bear in mind that fibre type classification on the basis of the relative mitochondrial volume, Z-band width and volume density of lipid droplets alone or in combination is unreliable as the overlap of these parameters between different fibre types is too great.

**STEREOLOGICAL DATA – RESPONSE TO TRAINING (II, V)**

**Endurance exercise.** Analysis of variance of the ultrastructural parameters studied here showed that apart from \( V_{\text{mit}} \) significant interaction existed between individuals and fibre types in endurance trained subjects. These results allow a number of important conclusions to be drawn. One cannot regard the fibre populations as homogeneous i.e. the inter-individual variations are so large that one must study the individuals separately. The results also show that endurance training reduces the difference between individuals
Vmit in all fibre types was higher in the endurance trained individuals (cf 53, 63, 83, 101), also differences in Vmit between Type 2A and Type 2B fibres were minimal or absent in biopsies from either trained or untrained individuals. No difference in oxidative capacity between Type 2A and Type 2B fibres was found in a parallel biochemical study on comparable material (33). This further strengthens the assertion that fibre typing on the basis of Vmit is doubtful, especially as far as subtyping of Type 2 fibres is concerned.

Lipid content tended to be higher in all fibres in trained individuals than in the untrained ones. However, only the Type 2A fibres showed definite differences between the two classes. It is thought that fibres that are exposed to high blood flow have a higher lipoprotein lipase activity and thereby higher lipid content (86). Cytological studies by Gauthier and Padykula (38) and Fiehn and Peter (34) have revealed a higher lipid content in Type 1 fibres. On the other hand, exhaustive work has been shown to induce a reduction in the amount of lipid, the reduction being more extensive in Type 1 fibres than in Type 2 fibres (93). This is another possible explanation for the undramatic differences in the lipid content in the muscle fibres in study II (biopsies taken immediately after finished long distance running) between trained (runners) and untrained individuals.

Z-band width is similar in the two groups when studied by stereological techniques. However, it is conceivable that an increase in synthesis in Z-band proteins and cytoskeletal proteins occurs as a response to training.

**Eccentric exercise — prolonged training (V).** Training caused no dramatic changes in the structure or occurrence of subcellular components though one should keep in mind that the biopsy was taken after a month of training. Accordingly, any changes within the fibres prior to the biopsy are unknown. It is probable that some myofibrillar disruption occurred during the first two weeks of training as performance was decreased during this period. No measurements of myofibrillar volume density were made though it is unlikely that this increases. Only a moderate improvement in concentric strength and no change in the average fibre size was seen after training.

A sign of adaptation is an increase in the mitochondrial volume fraction during the first half of the training program followed by a decrease. This is interpreted as an improvement in physical coordination resulting in fewer motor units being activated (26). This assertion also suggests that oxygen consumption is not increased despite successive increase in load and that a displacement to an increased Type 2B recruitment (shown by PAS-staining) was a result of the training.

**FUTURE PROBLEMS AND PROSPECTS IN PERSPECTIVE**

The methods used in the present work to induce muscle soreness and the ability to distinguish muscle fibres of different types at the ultrastructural level open up many possible directions for further study of the adaptability of human fibre populations to work involving high tensions. It is important to elucidate the significance of the size of load and duration of work, particularly from a work environment point of view. Does fibre necrosis occur with higher load in humans as studies by Armstrong and coworkers (6) have shown that eccentric work in rats can give rise to lethal fibre injuries. The satellite cell population is very sensitive to muscle fibre damage and show increases in number and evidence of "activation" even when the muscle fibre is gently compressed such that no degeneration occurs (115). The possibility of using this cell as a marker for more subtle changes within the muscle fibre will be investigated. Also changes in the distribution and organization of intermediate filaments in sore muscles are being investigated by more sensitive techniques involving immunofluorescence of semithin sections and immunoelectron microscopy.

Is the frequency of myofibrillar injury different between Type 1- and Type 2-dominated muscles under comparable loads? It is also considered important to investigate whether repeated concentric contractions give rise to the same damages as eccentric work. Can training with eccentric contractions induce sarcomerogenesis and in this way increase the functional length of the muscle and consequently modify the muscle power? The results from an extended study on long term training with eccentric contractions indicate that this indeed is the case (unpublished observations). The mechanism seems to be a longitudinal splitting of the Z-band.

What role do the increased number of Type 2C fibres play after eccentric training? Does this form of training change the contraction characteristics of fibres such that a fibre type transformation take place? There are many questions but still few answers. Studies in progress, based on the present findings, are designed to throw further light on the structural and functional adaptability of human skeletal muscle fibres exposed to increased tension demands.
GENERAL SUMMARY AND CONCLUSIONS

- Repeated eccentric contractions give rise to a delayed onset of stiffness, weakness and pain in the muscles utilized. Pain is aggravated with active movements. The discomfort lasts about 2 to 5 days.
- Muscle weakness corresponds to an objective strength reduction which last over the period the symptoms are apparent.
- Strength reduction is most pronounced and most persistent with rapid contractions.
- Myofibrillar disturbances are found in biopsies taken from sore muscles. The changes are most extensive when the symptoms are most pronounced i.e. 2–3 days after the exercise. The morphology is restored after 6 days.
- Ultrastructural fibre type classification based on the results from stereological measurements shows many sources of error. Accordingly, volume density of mitochondria and lipid droplets exhibit a large overlap between different fibre types (particularly between Type 2A and Type 2B). Physical training also influences fibre type discrimination validity.
- The fine structure of the M-band is a useful parameter for differentiating fibre types.
- The Z-band is, morphologically, the most sensitive myofibrillar component with eccentric exercise. Type 2 (fast-twitch) fibres were particularly affected in the present study.
- Mechanical overload caused by the high tension that is developed in eccentric exercise is judged to be the primary reason for the myofibrillar disorganization. The Z-band appears to be the weak link in the chain of contractile units.
- Eccentric muscle training reduces the risk for muscle soreness, improves the strength (eccentric as well as concentric dynamic strength) and reduces the risk for myofibrillar lesions.
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LITERATURE CITED


