HUMAN SKELETAL MUSCLE ULTRASTRUCTURE

ULTRASTRUCTURAL EXAMINATION OF HUMAN TYPE I AND TYPE II FIBERS

By

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ABSTRACT

It is known that human skeletal muscle has several fiber types, each with a different metabolic and physiological profile. It is not known whether there are different ultrastructural characteristics within each fiber type which may account for the different contractile properties. The purpose of this study was to examine the relationship between fiber type and ultrastructural characteristics.

Needle biopsies were obtained from the right vastus lateralis of five healthy males. According to the method described by Alway et al (1980), the tissue was prepared for electron microscopy and fiber types were identified based on their myoglobin content. Three non-overlapping electron micrographs were taken from the interior of each fiber under a Philips 300 electron microscope, and 50 type I and 50 type II fibers were examined from each subject. The constituents which were studied included: myofibrillar protein, mitochondria, cytoplasm, lipid and sarcoplasmic reticulum (SR). Volume densities were calculated for each constituent by means of a 168-point counting technique from electron micrographs projected onto a screen.

The results indicated that type I fibers were significantly greater with respect to mitochondrial volume density and lipid volume. However type II fibers had significantly greater myofibrillar volume densities and sarcoplasmic reticulum volume density (by approximately twofold). Fiber areas were also greater in type II than type I fibers while myosin packing density did not differ between fiber types.

It was concluded that SR and myofibrillar protein ultrastructural differences between fiber types may be important factors in determining twitch contraction properties and tension production for type I and II fibers. Finally, differences in mitochondrial volume density may be important in determining fatiguability of fiber types.

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CHAPTER 1

1. Introduction

A. Purpose of Study

It is known that there are three major fiber types in human skeletal muscle and that each fiber type has a different metabolic and physiological profile. However, the extent to which differing structural characteristics between fiber types might account for different contractile properties is not known. This lack of detailed knowledge as to ultrastructural differences in human muscle with respect to fiber type can largely be attributed to the lack of a suitable technique for determining fiber types for electron microscope analysis. The purpose of this study was twofold: (1) To develop such a technique, and (2) To apply it to the examination of the ultrastructure of different fiber types in human skeletal muscle.

B. Rationale

It is necessary to use ultrastructural and histochemical techniques in order to obtain maximal information about the properties of different fiber types. Various authors have attempted this process with varying degrees of success. Several workers have separated a muscle biopsy specimen into several smaller parts and subjected each to a different method of analysis (Gollnick et al, 1973; Costill et al, 1976; Thorstensson et al, 1976). Other methods have involved serial sectioning of a specimen and subsequent treatment of serial sections with a different method of analysis (Brooke and Kaiser, 1970; Edström and Ekblöm, 1972; Andersen, 1975). This procedure allows examination of the same fiber after various histochemical procedures. Ingjer (1977) described a method involving the freezing of non-fixed tissue for fiber typing prior to ultrastructural analysis. It is known that this may cause

freezing artifacts, which would invalidate the results.

Attempts have been made to classify fiber types at the ultrastructural level based on M and Z line widths (Payne, 1975) or mitochondrial size and volume densities (Cullen and Weightman, 1975; Van Winkle and Schwartz, 1978a). Such methods require longitudinal sectioning and are thus impractical for cross-sectional analysis. Furthermore, use of these methods on tissue obtained by the needle biopsy technique may be invalid since the muscle contracts immediately after excision.

Material processed for electron microscopy is generally unsuitable for histochemical analysis because most enzymes are inactivated by the fixative. Conversely, tissue which has been prepared by standard procedures for histochemistry involving cryostat sectioning prior to fixation for electron microscopy shows distortion at the ultrastructural level as a result of the freezing process.

Ultrastructural properties of different fiber types have been studied by comparing fibers from different muscle groups (S hafiq et al, 1969; Tomanek, 1973; Eisenberg et al, 1974; Eisenberg and Kuda, 1975; Van Winkle and Schwartz, 1978a). The authors assumed that all of the fibers within the sampled muscle were either FT or ST. This may not have been the case.

It is known that skeletal muscles differ anatomically in fiber lengths, origins and insertions, fiber arrangement and use. Burke et al (1974) have observed that the same fiber type (ST) from gastrocnemius and soleus (SOL) muscles have distinctly different physiological profiles. Since the physiological profiles from the same fiber type of different muscles are dissimilar, it is likely that the ultrastructural profiles will also be different. Therefore it may not be valid to compare the ultrastructural properties of

fiber types from different muscles. The most valid method is to compare ultrastructural characteristics according to fiber type within the same area of one muscle and relate this to the contractile properties of that muscle. This would assume that the ultrastructural properties are homogeneous for a given fiber type within a single muscle. Therefore this study used a method which allowed the comparison of the ultrastructural profiles of type I and II fibers within the same muscle based on their myoglobin content and with minimal freezing artifacts. Ultrastructural (i.e. stereological) analysis was conducted in order to obtain normative stereological data for each fiber type.

C. Hypothesis

There are structural differences between type I and II fibers which may partially account for the differences which are known to exist with respect to contractile properties of different fibers.

C.1 Subhypothesis

Sarcoplasmic reticulum and transverse tubule volume densities will be significantly more extensive in type II fibers.

It has been observed from animal muscle that FT fibers have both greater tension output per unit cross sectional areas and shorter twitch contraction times. Further studies have shown correlations between greater calcium release and uptake and twitch time and tension. Since the SR network is responsible for the release and uptake of calcium, it is the contention of this study that there will be a greater SR volume density in type II fibers.

D. Limitations

1. In order to examine the difference between type I and II fibers the

vastus lateralis muscle was used since it contains both fiber types. The results therefore pertain only to the vastus lateralis muscle.

2. The conclusions are limited to healthy active but non-trained male students aged 20-21. These subjects are considered to be representative of a normal population as opposed to trained athletes or persons with neuromuscular disease.
3. This study is based on fiber typing according to myoglobin localization which has been validated by a modified Brooke and Kaiser (1970) ATPase reaction. It is assumed that the ATPase method is valid and reliable for fiber typing of human skeletal muscle.

2. Review of Literature

Muscle fiber investigations have spanned many years of research from early muscle categorization by fiber color to more recent advances in technology with physiological, histochemical and ultrastructural studies.

Considerable confusion exists according to different classification techniques based on different histochemical, biochemical, physiological and morphological properties and from the attempt to relate these characteristics to either whole muscle or single muscle fibers. As a result, several systems have been reported which describe various muscle types.

In the following review pertinent literature related to the classification of muscle fiber types is discussed according to (a) color characteristics, (b) fiber nomenclatures, (c) twitch characteristics, (d) histochemical properties, (e) biochemical properties, (f) physiological properties and (g) ultrastructural properties. Literature is then summarized as to the physiological significance of myoglobin and finally, studies dealing with the possibility of fiber-type conversions are discussed.

I. Classification of Fiber Types

1.IA Color Characteristics

The oldest classification of fiber types was based on appearance. In 1878 Stephani Lorenzini observed that animal muscle could be subdivided into red and white muscle on the basis of color (cited in Dubowitz, 1965a). Such classification of whole muscles being red or white occurred despite the fact that some fibers of the opposite color appeared within the muscle. Since that time scientists have learned that it is the myoglobin content of the muscle fibers which give the characteristic color to the muscle.

Fiber types based on color characteristics have been expanded to include intermediate fibers (Padykula and Gauthier, 1966). Many morphological and cytochemical (intensity of enzyme reactions) criteria have been used along with color differentiation to distinguish the fiber types.

Padykula and Gauthier (1966) described red fibers of the rat as having the smallest diameter (27μ), being rich in mitochondria and having a high mitochondria to myofibrillar mass ratio. The white fibers had the largest diameter (44μ) and few mitochondria (Padykula and Gauthier, 1966). The same authors described an intermediate colored fiber, which had an intermediate diameter (34μ) and intermediate mitochondrial content. Early biochemical analysis showed red fibers to be high in SDH while white muscles were low in SDH (Padykula, 1952).

1.IB Fiber Nomenclatures

"Red" and "white" nomenclatures are too inaccurate to describe or infer muscle type and metabolism (Peter, 1973). Romanul (1964) devised an elaborate classification of rat muscle fibers. He included three major groups

and a total of eight subgroups (I: A, B and C; II: D and E; and III: F, G and H). He described group II as having the greatest oxidative activity and group III as moderate oxidative activity. His group II fibers correspond to red fibers. More confusion arises when these oxidative enzyme classifications are used interchangeably with the more popular type I and II classification based on M-ATPase staining (Brooke and Kaiser, 1970).

No system of classification is universally accepted. More widely accepted animal nomenclatures are from the work of Peter et al (1972) or Burke et al (1971). Peter et al (1972) described physiological and biochemical properties by using the terms fast-twitch glycolytic, and slow twitch oxidative. Burke et al (1971) used a different physiological property and classified fibers as fast fatiguing, fatigue resistant or slow fibers. Among the most widely accepted classification for human muscle is based upon a nomenclature originated by Engel (1962) and modified by Brooke and Kaiser (1970). The nomenclature of the latter authors uses type I, IIA, IIB or IIC fibers. Type I and II fibers can be differentiated on the basis of their histochemical (Brooke and Engel, 1969; 1969a; Brooke and Kaiser, 1970), metabolic (Taylor et al, 1974; Essen et al, 1975), physiological (Buchthal and Schmalbruch, 1970; Sica and McComas, 1971; Warmoltz and Engel, 1972; Schwart et al, 1977), morphological (Polgar et al, 1973) and ultrastructural profiles (Tomanek et al, 1973; Payne et al, 1975). Type II fibers can be subdivided histochemically (Brooke and Kaiser, 1970). The IIC fiber represents a very small percentage of most human muscle (Ringvist, 1973; Dubowitz and Brooke, 1973; Jansson and Kaiser, 1977), but a greater percentage has been reported in the muscles of some athletes (Jansson and Kaiser, 1977). In the following discussion of properties of different fiber types the Brooke and Kaiser (1970) classification system

will be used.

1.IC Myosin Chains

It is generally accepted that myosin from animal FT and ST muscle is structurally and immunochemically different (Sréter et al, 1966; Lowey and Risby, 1971; Arndt and Pepe, 1975; Gauthier et al, 1978; Lutz et al, 1978).

Myosin chains have been classified according to their molecular weights (as "heavy" or "light" chains) and M-ATPase activity (as fast or slow). The heavy chains comprise about 85-90% of the myosin and run the entire length of the myosin molecule. The light chains make up the final 10-15% of the myosin, and are confined to the head region of the molecule (Lowey and Risby, 1971; Starr and Offer, 1973).

Type I fibers have only slow myosin (SM) while type IIB fibers had only fast myosin (FM) (Rubinstein et al, 1979; Lutz et al, 1979). Conflicting results have been found for type IIA and IIC fibers. Rubinstein et al (1978) did not find any type II fibers which contained both FM and SM. However, Lutz et al (1979) did observe varied proportions of both FM and SM in type IIA and IIC fibers. Lutz et al (1979) argued that their method of combining classical gel electrophoresis with an enzyme linked immunological method provided a much more sensitive approach to identifying myosin chains.

1.IC.1 Fast Myosin Light Chains

Three light chains of FM have been identified in skeletal muscle. The light chains have molecular weights of 25,000, 18,000, and 16,000 and are classified LC^f_1, LC^f_2 and LC^f_3, respectively.

One class of FM chains is liberated by treatment with 5,5' dithiobis (DTNB) and is designated as the DTNB light chains. This is the LC^f_2 chain (Gauthier and Lowey, 1979). The other class is removed by exposure to alkaline pH and have been termed alkali light chains (Weeds et al, 1975). The larger alkali 1 chain (LC^f_1) is similar to the smaller alkali 2 chain (LC^f_3) except that the alkali 1 has an additional 41N-terminal residues. These extra residues account for the greater molecular weight (Gauthier and Lowey, 1979).

Recently Hoh and Yeoh (1979) have identified three different components of fast myosin (FM_1, FM_2, FM_3). FM_1 has two chains each of LC^f_2 and LC^f_3 (ratio of 1:1). FM_2 has all three light chains: one chain of LC^f_1, two chains of LC^f_2 and one chain of LC^f_3 (ratio of 1:2:1). FM_3 has two light chain of each LC^f_1 and LC^f_2 (ratio of 1:1). LC^f_1 is absent in FM_1 and LC^f_3 is absent in FM_3. All three components of fast myosin have a total of four light chains each.

1.IC.2 Slow Myosin Light Chains

Only two light chains with molecular weights of 27,000 (LC^s_1) and 20,000 (LC^s_2) have been identified in slow myosin (SM) (Lowey and Risby, 1971; Weeds et al, 1975; Pelloni-Mueller et al, 1977; Gauthier and Lowey, 1979; Hoh and Yeoh, 1979). SM has equimolar amounts of LC^s_1 and LC^s_2. In addition to missing a 16,000 molecular weight subunit, the slow muscle myosins have light chains of slightly higher molecular weight than those of the FM (Lowey and Risby, 1971).

The classes of SM have not been studied as extensively as FM. One class known to exist consists of two chemically similar light chains which are related to the alkali light chains of FT muscle. The second class has some properties in common with the DTNB light chain (Gauthier and Lowey, 1979).

1.IC.3 Myosin Heavy Chains

Myosin from either FT or ST muscle consists primarily of two heavy chains of myosin, each with a molecular weight of 200,000 (Lowey and Risby, 1971; Weeds et al, 1975, Pelloni-Mueller et al, 1977; Suthoh et al, 1978; Gauthier and Lowey, 1979; Hoh and Yeoh, 1979). The heavy chains have slightly different peptide arrangements and therefore are separate and distinct heavy chains (Starr and Offer, 1973).

The heavy chains make up most of the myosin molecule, but both the heavy and light chains intertwine to form the myosin structure. Along approximately one-half of their length, the heavy chains form a linear coil of parallel α-helixes while the remainder of each heavy chain folds separately into a globular region which together with the light chains forms each of the two heads of the myosin molecule (Sutoh et al, 1978).

Heavy chains of FM and SM have been reported to be structurally different. Jean et al (1975) observed tryptic digestion of myosin from FT and ST muscles of the rat revealed a distinct peptide band (molecular weight of 88,000) in FM which was absent in SM.

The authors found that the light chains do not influence this peptide pattern of fast and slow muscle, since there is still a unique peptide chain in FM when all the light chains were removed. The authors concluded that there are differences in FM and SM heavy chains and suggested that two separate genes were responsible for synthesizing of the heavy chains.

Arndt and Pepe (1975) observed two antigenically distinct heavy chains of myosin from chicken ALD (red) and PLD (white) muscle. Hoh and Yeoh (1979) also found distinctly different patterns of cyanogen bromide mapping for myosin heavy chains (from rabbit PLD and SOL muscles) and con-

cluded that heavy chains from FM and SM are structurally different.

1.IC.4 Myosin Chains During Development

Light chains of fetal and adult skeletal muscle have the same configuration and molecular weights (Sréter et al, 1975; Takahask and Tomomura, 1975; Pelloni-Mueller et al, 1976; 1977; Hoh and Yeoh, 1979). In contrast, fetal and adult heavy chains are structurally different (Hoh and Yeoh, 1979).

1.IC.4a Slow myosin development

It is known that there are developmental changes in contractile properties of muscle from the embryonic stage to old age. The M-ATPase activity of animal SOL before birth is greater than that of the adult (Syrový and Gutmann, 1977). Rabbit embryo SOL myosin has a predominance of light FM chains (Syrový and Gutmann, 1977). During post-natal development the amount of light chains of FM decrease while SM light chains increase, until as an adult the rabbit has only light chains of the slow type in the SOL muscle (Syrovy and Gutmann, 1977). This was also shown to occur in chicks (Sreter et al, 1975).

1.IC.4b Fast myosin development

Fast myosin synthesized in animal embryos has the same light chain complement as adult myosin except a lesser amount of LC^f_3 and a slightly lower ATPase activity (Sréter et al, 1975). During post natal development an increase in LC^f_3 is closely associated with an increase in M-ATPase activity (Takahasi and Tonomura, 1975).

1.IC.4c Synthesis of myosin isoenzymes during development

There exists some controversy regarding the synthesis of myosin isoenzymes during development. Gauthier et al (1978) demonstrated that significant amounts of slow as well as fast isoenzymes of myosin were present

during early development in FT muscle (both isoenzymes co-existing in the same fiber). Syrovy and Gutmann (1977) found that the SOL muscle possessed fast light chains before birth but the fast chains disappeared after birth. Likewise the ATPase activity decreased in the SOL muscle as the animal matured. The authors also suggested that fast and slow myosin exist initially in the same muscle.

More recently Hoh and Yeoh (1979) and Rubinstein and Holtzer (1979) showed that fetal muscle synthesized only fast myosin (both heavy and light chains) before normal innervation. Subsequently after innervation as the embryo matured, the motor neurons were thought to overide this endogenous occurance and the fetal myosin enzymes were replaced by the appropriate adult FT or ST isoenzymes.

1.IC.5 Myosin Subunit Functions

There is presently little known about the function of the specific myosin subunits. Wagner and Weeds (1977) hypothesize that the LC^f_2 subunit may be involved in a calcium regulatory mechanism associated with muscle contraction. The other two light chains (LC^f_1 and LC^f_3) were thought to be involved in regulating the level of myosin Ca^{2+} dependent ATPase activity (Dreizen and Gershman, 1970; Dow and Stracher, 1971). However, Wagner and Weeds (1976) found that the alkali light chains (LC^f_1 and LC^f_3) did not influence the ATP turnover, but affected the actin activated ATPase activity.

There does not appear to be any selectivity of interaction between the myosin chains since both types of heavy chains are associated with each type of alkali light chain (Pope et al, 1977).

It is not yet known what differences exist between the function of the various subunits of fast and slow muscle myosin.

1.ID Histochemical Properties

Histochemistry allows a qualitative evaluation of enzymatic properties of cells. Enzymes which reflect the metabolic properties of different fiber types are localized within the muscle cell. Consequently, the intensity of the histochemical stain indicates the activity of that particular enzyme in that cell. Fiber typing involves comparing the intensities of enzymatic reactions between the muscle fibers.

Three major enzymatic groups are used in classifying fiber types. The first group is that of the glycolytic enzymes which are enzymes used in the production of adenosine triphosphate (ATP) from the Embden-Meyerhof pathway (McGilvery, 1975). Such "anaerobic" metabolic pathways do not require the presence of oxygen to form ATP (McGilvery, 1975). The two enzymes in this pathway which are most commonly assayed are alpha glycerophosphate dehydrogenase (αGPD) and phosphorylase.

The second group are the oxidative enzymes which are critical in the electron transport and citric acid cycle of aerobic metabolic pathways (McGilvery, 1975). Typical oxidative enzymes measured include succinate dehydrogenase (SDH) and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) (Brooke and Kaiser, 1970; Engel, 1974; Askanas and Engel, 1975). Type IIA and IIC fibers have been reported as being higher in oxidative enzymes and lower in glycolytic enzymes than type IIB fibers (Engel, 1974; Askanas and Engel, 1975).

The final group of enzymes are the adenosine triphosphatases (ATPase) which are essential in the contraction process by removing a phosphate group from adenosine triphosphate (ATP) to form adenosine diphosphate (ADP) plus an inorganic phosphate. This step allows the release of energy which is

necessary for the binding of actin and myosin for muscular contraction (McGilvery, 1975). Barany (1967) observed that the speed of muscle contraction was directly proportional to its myosin ATPase activity. As already discussed, two different myosins have been found in muscle. This is the basis for fiber type differentiation (Guth and Samaha, 1969). Type I fibers are acid stable and stain lightly at an alkaline pH of 9.4 and darkly at an acid pH of 4.3 (Brooke and Kaiser, 1970; Engel, 1974; Askanas and Engel, 1975). Type II fibers are generally alkaline stable and stain the opposite of type I fibers. There are, however, variations in staining intensity at various pH levels which further differentiate type II fibers. Type IIA, IIB and IIC stain darkly at pH 9.4. Type IIA and IIB have no reaction at a pH of 4.3, while type IIC has a moderate reaction. At a pH of 4.6 both type IIB and IIC stain strongly, but type IIA has no reaction (i.e. M-ATPase is inhibited) (Brooke and Kaiser, 1970).

1.IE Biochemical Properties

Muscle enzyme activity (biochemical analysis) has been compared to the percentage of different fiber types in human muscle. Gollnick et al (1974) compared phosphofructokinase (PFK), creatine phosphokinase (CPK) and phosphorylase from muscle homogenates with known percentages of type I (ST) fibers in the human vastus lateralis, gastrocnemius and soleus muscles. Both the vastus lateralis and gastrocnemius muscles had 57% type I fibers (range 34-82%) while the soleus had 80% type I fibers (range 64-100%). Biochemical analysis showed that glycolytic enzymes (excluding hexokinase) were lower in muscles with a predominance of type I fibers and higher in muscles with greater type II percentages. SDH and CPK were not found to be related to

the percentage of fiber type (Gollnick, 1974; Thorstensson et al, 1976). This type of analysis is useful to show relationships between fiber type distribution and biochemical properties of whole muscles but has obvious limitations. A more accurate method has been introduced by Essen et al (1975) where individual fibers were separated from freeze dried muscle samples by a process of micro-dissection. Staining for ATPase activity was performed on the end segment of each fiber. This allowed fiber type identification. The middle section of each fiber was weighed then assayed for glycogen, triglycerides, sodium and potassium contents. Biochemical analysis could be conducted on individual or grouped fibers once the fiber type was known. The oxidative enzymes SDH and NADH-TR reaction were found to be strong in type I, moderate in type IIA and IIC and weak in type IIB fibers. Glycolytic enzymes GPD and PFK had a weak type I, moderate IIA and IIC and strong IIB reaction. Biochemical assays for myosin ATPase activity found that type I was low while the remaining type II fibers were all high. The reverse was observed for triglyceride content. Glycogen concentration was similar for all fiber types (Essen et al (1975), however, there was a several hour delay between fiber preparation for dissection and biochemical analysis.

Thorstensson et al (1977) also used the micro-dissection method to obtain activity levels of CPK, myokinase (MK), lactate dehydrogenase (LDH) and Mg^{2+} stimulated ATPase in human type I and II fibers. Type II fibers showed three times the level of Mg^{2+} stimulated ATPase than did type I fibers. LDH and MK were also higher in type II fibers but CPK did not show a significant difference between fiber types. These two studies show that there are significantly different biochemical and histochemical differences between fiber types.

1.IF Physiological Properties of Fibers

1.IF.1 Twitch Times

A muscle twitch is a contractile response of either whole muscle or a single motor unit to a single stimulus. The study of twitch characteristics was useful to determine differences between muscles.

Physiologists have long been aware that muscles could be categorized as slow or fast depending on twitch contraction times. Late in the 1800's Ranvier observed that red muscle contracted more slowly than did white muscle (cited in Close, 1967). It is known that a muscle is not "born" as fast or slow twitch. Close (1967) observed EDL and SOL muscles in the rat at various stages of development. He observed that isometric twitch characteristics were the same for both EDL and SOL at birth. Responses became faster for EDL as the animal matured leading to a fast muscle as an adult. Twitch times for SOL decreased slightly at first then increasedas it differentiated to a slow muscle.

The usually accepted theory that red muscles have high oxidative enzyme activity and slower contraction times than white muscles which have high glycolytic enzyme activity was questioned by Hall-Graggs (1968). He obtained the thyroanthenoid muscle from the rabbit and found that it has extremely fast twitch characteristics but is histochemically "red". All the muscle fibers stained high in SDH. However, SDH was later found to be unreliable method for fiber differentiation (Gollnick, 1974; Thorstensson, 1976a). Similarly, Barnard et al (1971) found that some "red" rat muscles had fast contraction times. He concluded that red fibers could either be fast or slow twitch. Neither Hall-Craggs (1968) nor Barnard et al (1971) considered an intermediate fiber which would have accounted for their observations. The distinction between fast and slow muscle is based on twitch differences between muscles within a particular animal. In general, the muscles from smaller animals have faster twitch times than corresponding

muscles of large animals (Close, 1967).

Actual contraction times are therefore not a valid basis for determining muscle types between animals. Muscles of animals of different sizes and shapes have approximately the same intrinsic tensile strength whereas intrinsic speed varies approximately as the inverse of the linear dimension of the animal (Hill, 1950). The ratios of speeds of fast and slow muscles are the same in different mammals. Fast muscles have isometric twitch contraction times one-half to one-third the value of slow muscle (Close, 1967). Sica and McComas (1971) observed fast (35-74 ms) and slow (78-90 ms) motor units in human extensor hallucis brevis (EHB) muscle. Similarly, Buchtal and Schmalbruch (1970) found long contractions in soleus and gastrocnemius muscle (60 msec) and shorter cnntraction time (msec) in bicep brachii and triceps brachii muscles. There is a strong direct relationship between changes in intrinsic speed of shortening of the sarcomere and myosin ATPase activity (Bárány, 1967; Close, 1972). This was confirmed by Burke et al (1971) who observed myofibrillar ATPase staining correlated well with the speed of shortening of individual motor units. This supports the hypothesis that the rate of shortening is limited by the rates of ATP hydrolysis by myosin at individual enzyme sites (Close, 1972).

1.IF.2 Correlations of Physiological and Histochemical Properties

Early attempts to correlate physiological and histochemical properties of mammalian muscles depended upon indirect evidence based on properites of a whole muscle (Padykula and Gauthier, 1966). However most muscles are composed of a combination of different fiber types. The muscle fibers making up a skeletal muscle are organized under the functional entity

of a motor unit. The motor unit consists of a group of muscle fibers and the single motor neuron innervating them.

1.IF.2a Fatiguability in Skeletal Muscle

Fatiguability of motor units are closely associated with the histochemical type of its fibers (Edström and Kugelberg, 1968). Repetitive low frequency (5/sec) electrical stimulation of single motor units in rat tibial muscle was shown to cause a rapid decline in both twitch and tetanic tensions in type A units (i.e. type IIB fibers) and they became fatigued in five minutes. Some type B fibers (i.e. type IIA fibers) fatigued within ten minutes at greater frequencies (10/sec) but other units continued to function. Type C units (i.e. type I fibers) did not show any decline in twitch tension after several hours of repetitive stimulation of 10/sec. It was concluded that type A units were very fatiguable while type C units are fatigue resistant and type B units have an intermediate fatigue resistance (Kugelberg and Edström, 1968).

Burke et al (1971) were able to classify three different motor units from the cat gastrocnemius muscle by twitch time and fatiguability. They were: fast contracting, fast fatiguing (FF); fast contracting, fatigue resistant (FR); and slow contracting (S) units. The recent work of Garnett et al (1979) confirmed the existence of the three motor units in human gastrocnemius muscle. These authors found that FF units were composed of type IIB fibers while FR and S units were composed of type IIA and type I fibers respectively. These results agree with the earlier findings of Thorstensson and Karlsson (1976) who observed a positive correlation ($r = 0.86$, $p < .01$) between fatigue (percentage of decline of force over fifty contractions) and the percentage

of FT fibers in the vastus lateralis of human subjects.

The fatiguability patterns also influence the recruitment patterns of fiber types. During graded and voluntary muscle contractions motor units are recruited in order of increasing contraction strength and lessening fatigue resistance (Stephens and Usherwood, 1977). Consequently, type I and IIA muscle fibers would be employed at low levels of force (and could be maintained for a sustained time), followed by type IIB fibers in stronger contractions (but for a shorter sustained contraction because of a high fatiguability) (Milner-Brown et al, 1973). In summary, type IIB fibers fatigue quickly, type IIA fibers have an intermediate rate of fatigue and type I fibers fatigue very slowly.

1.IF.2b EMG Data and Fiber Types:

Single fiber EMG recordings and histochemical data have been obtained in patients with various neuromuscular diseases (Schwartz and Dubowitz, 1977). The authors were only able to make general correlations between the contractile properties (twitch characteristics and histochemical data (M-ATPase), because they could not determine if the EMG was recorded at the precise site along the fiber from which the biopsy was taken.

Warmolts and Engel (1972) studied the EMG activity of human biceps brachii and quadriceps muscle in areas of almost completely one fiber type by an open biopsy technique. This allowed the EMG results and the muscle fiber type (based on M-ATPase reaction) to be compared from the same area. The authors observed that type I muscle fibers displayed: (1) activation after minimal to mild voluntary contraction; (2) steady rhythmicity on low effort contraction at discharge frequencies ranging from 6-10/s; (3) sustained discharges through all ranges from mild to maximal contraction; and (4) with increasing effort of contraction an increase in

firing frequency, reaching a maximum of 18-20/s on full voluntary effort.

Type II muscle fibers had characteristics of: (1) activation occurring only after sudden or brief vigorous contractions; (2) they either discharged in brief bursts, lasting 0.5 to 5 seconds with the inability to sustain contractions any longer, or the units discharged only once or twice; (3) peak firing frequencies ranged from 16 to 50/seconds and were reached during the most vigorous contractions; and (4) motor unit potentials successively recruited tended to have a greater force of contraction and had more rapid peak discharge frequencies.

Buchthal and Schmalbruch (1970) studied contraction time and fiber types in human adductor pollicis muscles, and found that contraction time was related to fiber type. Intact fast-twitch muscle had a contraction time less than 60 ms and slow muscle was greater than 60 ms. Type I fibers (determined by mitochondrial content using Sudan Black stain) had long contraction times. Type IIB fibers and IIA fibers both had short contraction times. While these physiological observations fit quite well with histochemical determination, it should be noted that mitochondrial content has been observed to be a poor method of distinguishing fiber types when used exclusively, in the absence of other enzymatic reactions (Barnard et al, 1971). Moreover, the oxidative enzymes (which are related to mitochondrial content) such as SDH also apperas to give poor fiber type determination (Gollnick et al, 1974).

1.IF.2c Motor Unit Firing Rate and Recruitment

Firing rate and recruitment order of human toe extensors have been recently studied (Grimby and Hannerz, 1977). The toe extensor muscles were classified by an alkaline ATPase method and described as having 54% type I and 46% type II fibers. Subunits were not classified. They observed that

some motor units could be driven continuously and attain regular firing intervals even at a firing rate of 10/s. These motor units called continuously firing long interval motor units (c.l.m.u.s.) increased their firing rate with each contraction strength, but had a maximum firing rate less than 30/s during a sustained contraction. The c.l.m.u.s. could however attain rates of 60/s in a twitch contraction. The authors concluded that these continuously firing low frequency motor units represented type I fibers.

The second type of motor unit observed by Grimby and Hannerz (1977) was the intermittently firing short interval motor units (i.s.m.u.s.). These motor units could not be driven continusously. The i.s.m.u.s. did not fire at rates below 20/s and increased rapidly in firing rate with increases in contraction strength and attained firing rates above 100/s. These motor units were thought to correspond to type II fibers. There were intermediate firing forms between c.l.m.u.s. and i.s.m.u.s. These may have corresponded to an intermediate fiber and unit (i.e. type IIA). Such a classification would then name the i.s.m.u.s. as a IIB fiber and motor unit.

More recently, Grimby et al (1979) studied discharge properties of c.l.m.u.s. and i.s.m.u.s. motor units (by EMG) of the human toe extensor muscle during maximal voluntary contraction. They observed c.l.m.u.s. units to have long contraction times (60-90 ms) while i.s.m.u.s. had shorter contraction times (40-55 ms). The authors' findings of a relationship between voluntary discharge pattern and contraction time might be expected based on the observations of Salmons and Sreter (1976) that the discharge of the motor neuron may determine the contraction time of the muscle

fibers. Since no direct histochemical correlation was obtained, such as the open biopsy technique of Warmolts and Engel (1972), the claims associated with fiber type which are offered by Grimby and Hannerz (1977) and Grimby et al (1979) must be interpretated with caution.

1.IF.2d Histochemical and Biochemical Properties of Fiber Types:

However, the physical properties associated with the fibers do seem to coincide with the known biochemical profiles. For example, the type I fibers have a high oxidative capacity and a high triglyceride content for energy substrates (Essen et al, 1975; Askanas and Engel, 1975). This correlates well with the aerobic nature of the type I fiber and the observation that tension can be maintained in a continuous fashion at low frequencies (Grimby and Hannerz, 1977). The opposite would appear true of the type IIB fibers which have high glycolytic enzyme activities (Essen et al, 1975; Askanas and Engel, 1975) and presumably a greater glycogen content (Brooke and Kaiser, 1970; Engel, 1974: Askanas and Engel, 1975). The metabolism of such a muscle would appear quite suited for the intermittent bursts of high activity that Grimby and Hannerz (1977) describe for the type II fiber. Type IIA fibers are histochemically intermediate between type I and IIB. The intermediate firing characteristics overlapping both of the other fiber types appears to fit quite well with such a histochemical profile. Fiehn and Peter (1971) reported guinea pig fast-twitch white (FTW) fibers to have the shortest contraction and relaxation time, then the slow twitch soleus had the longest contraction time and an eightfold greater half relaxation time than FTW fibers. However, it must be noted again that such conclusions are as yet unsubstantiated by other researchers. More

valid histochemical identification of fiber types associated with motor unit firng rates will add valuable information in this regard.

1.IF.2e Tension Production in Motor Units

Burke and Tsairis (1973) have reported S unit muscle fibers in the cat to have smaller tension outputs per unit area (0.6 Kg/cm^2) than either FF units (1.5-2.0 Kg/cm^2) or type FR units (2.6-2.9 Kg/cm^2). However, unit tension output comparisons between different muscles are not valid. Homsher and Kean (1978) observed that the cat soleus S units produced more tension per unit area (2 Kg/cm^2) and had slower contraction times (97.1 ms) than did the S units of the gastrocnemius (0.6 Kg/cm^2 and 75.8 ms respectively).

Burke et al (1974) had earlier found similar physiological differences and concluded that the S units of the cat soleus and gastrocnemius were not the same despite both being slow twitch and resistant to fatigue. It is not known whether there are ultrastructural differences between the cat soleus S fibers and gastrocnemius S fibers (Burke et al, 1974). Tetanic tension of the rat tibialis anterior has been reported to be 3.6 Kg/cm^2 (Howells et al, 1978) but the fiber composition of the muscle or tension output for each fiber type was not calculated.

Strength and tension measures have been studied in animal and more recently intact human muscle. Gonyea and Bonde-Petersen (1977) found that the forelimb and hindlimb muscles of the cat had similar speed of contraction. The gastrocnemius and plantaris had a greater isometric twitch tension (strength production) than the slow twitch soleus muscle. The forelimb fast twitch muscles produced more tetanic tension per gram of muscle weight than the hindlimbs. However, the fast twitch hindlimb

had a greater rate of tension development. This was attributed to the greater proportion of fast twitch glycolytic fibers in the hindlimb (56%) compared to the frontlimb (38%).

Bárány and Close (1971) reported EDL of the cat to have greater maximal twitch tension (0.513 Kg/cm^2) and maximal tetanic tension (3.0 Kg/cm^2) than the SOL muscles (0.332 and 2.095 Kg/cm^2 respectively). The authors hypothesized that the difference in maximal tetanic tension per unit cross sectional area of muscle was not due to differences in the number of filament cross bridges per half sarcomere because the myosin yield (and presumably the myosin content per gram of fresh tissue) is the same in normal EDL and SOL muscles.

Both muscle mass and the quality of muscle appear to be important in strength (tension) development (Ikai and Fukunaga, 1968; Haggmark et al 1978; Tesch and Karlsson, 1978).

Haggmark et al (1978) studied the cross sectional area of the human thigh at a biopsy point by the use of soft tissue X-ray technique and computed tomography. The largest muscle areas were recorded in subjects who were weight lifters. Type II fibers had a much greater area in weight lifters than other trained subjects. The mean fiber area in each specimen was highly correlated to the cross sectional area of the vastus lateralis muscle ($r = .9$, $p < .001$). A linear positive (but low) correlation was found between maximal isometric one leg strength and the relative distribution of type II fibers. This was significant at $p < .001$ (Tesch and Karlsson, 1978). However, Thorstensson (1976) found that there was no difference in isometric knee extension peak torque between persons with a high percentage of either type I or II fibers. These authors did observe significantly greater torque outputs from persons having a high

percentage of type II fibers during contractions with high joint velocities of 180°/s (although this is only about 25% of the maximal contraction velocity of the knee).

Recently MacDougall et al (1979) reported that both type I and II fibers hypertrophied, and were assumed to be active in the production of force for heavy resistance training, but type II fibers had a greater relative involvement (hypertrophy). However, FT fibers may be more responsive to the training, and not necessarily more active. Heavy resistance training has previously shown greater hypertrophy in type II than type I fibers (Edström and Ekblöm, 1972; Prince et al, 1976; Thorstensson et al, 1976).

1.IF.2f Effect of Calcium on Tension:

Previous research has studied the application of caffeine to tetanically contracting animal muscle (Taylor and Rudel, 1970; Rudel and Taylor, 1971). The authors observed that caffeine potentiates the release of calcium from the sarcoplasmic reticulum. It was suggested that the amount of Ca^{2+} released determined the tension produced (Weber, 1968; Taylor and Rudel, 1970; Moss et al, 1976). It is known that each stimulus in tetanus is associated with a Ca^{2+} release (Homsher and Kean, 1978). However, changes in the amount of Ca^{2+} released from the SR greatly increases the free ion concentration, but was hypothesized to have only a slight effect on the amount of calcium which is bound to the regulatory proteins. Moss (1979) observed that high constant Ca^{2+} levels around skinned frog anterior tibialis did not affect the sarcomere lengths less than 2.0μ. He concluded that the cross bridge interactions (therefore the sarcomere lengths) may be more important in tension production than the level of Ca^{2+} present. This has not as yet been directly studied at sarcomere lengths greater than 2.0μ.

Homsher and Kean (1978) reasoned that if Ca^{2+} release was inhibited with stretching the sarcomere, there would be a decreased twitch/tetanus ratio. This was not found to occur. The authors concluded that the decline in tetanic force with increased sarcomere length is caused by a decline in myofilament overlap and not from insufficient Ca^{2+} production. In addition, it is known that at any given sarcomere length a post-tetanic potentiation can occur. This suggests that upon stimulation there was enough calcium released to stimulate the Ca^{2+} binding sites (Homser and Kean, 1978). However, these authors did not consider other elements which might affect the twitch/tetanus ratio in stretched muscle (e.g. the series elastic component), and thus mask the influence of Ca^{2+}.

1.IF.2g Effects of Sex on Muscle Physiological Profiles:

Comparative analysis of male and female muscle fiber types, enzyme activities and physical performance in twins have shown substantial differences between sexes by some authors (Komi and Karlsson, 1978) but not by others (Bell, MacDougall and Howald, 1980). Komi and Karlsson (1978) reported that adult females had greater percentages of type II fibers but significantly less Ca^{2+} activated ATPase, CPK, phosphorylase and LDH than their male twin. They observed that males as compared to females demonstrated higher aerobic and strength performance capacities, faster rises in tension curves during a contraction and more slow twitch muscle fibers in skeletal muscle. It is not known how these small samples may represent the general population. The authors found that males had a greater percentage of slow fibers but were stronger. This was explained by larger muscle girths and larger FT fibers ($p < .001$) found in males.

1.IF.2h Fiber Efficiency and Adaption in Work:

Suzuki et al (1979) have reported that persons with a high percentage of FT fibers in the vastus lateralis (76% FT) had a greater efficiency of bike ergometer pedalling at 100 rpm (greater than 80% of VO_2 max.) than persons with a high percentage of vastus ST fibers (78% ST). There was no difference in pedalling efficiency at 60 rpm. Efficiency was a measure of $\dot{V}O_2$ uptake against external work cost (watts). However the authors did not consider that at higher work rates the FT fibers may have been contracting anaerobically (due to their biochemical endowment) therefore consuming little oxygen. $\dot{V}O_2$ is thus probably not an accurate method to assess work efficiency between fiber types.

Work induced hypertrophy produced an increase in myofibrillar contractile filaments (Seiden, 1976; Edgerton, 1976; MacDougall et al, 1976; 1979). Edgerton (1976) found an increase in maximal tetanic tension with hypertrophy but no increase in maximal twitch tension. However, Gonyea and Bonde-Petersen (1978) found an increase in both twitch and tetanic tension in weight-lifting cats, and Sale et al (1979; personal communication) observed a greater twitch tension in persons involved in heavy resistance training over controls. The number and size of myofibrils increase with strength training (Seiden, 1976; MacDougall, 1976) and thus the increased number of myofilaments would account for the increased contractile force of resistance trained muscle.

1.IG Ultrastructural Properties

1.IG.1 Fiber Sizes

Small animals generally have small fibers, but there is considerable size variation even within the same animal (guinea pig, standard deviation/mean = 16%) (Eisenberg and Kuda, 1975). FTW fibers are usually larger than either FTR or ST fibers (Tomanek, 1973; Eisenberg and Kuda, 1975; 1976). Type II fibers have been reported to be larger in rat anterior tibialis than type I fibers (Howells et al, 1978). Similarly, samples from biceps brachii, triceps brachii and vastus lateralis have indicated that type II fibers were larger in males than type I fibers (Brooke and Engel, 1969; Polgar et al, 1973; MacDougall, 1979) while the reverse is true for female muscle (Brooke and Engel, 1969). Polgar et al (1973) obtained autopsy samples from 50 different muscles in males. They observed 44 of the muscles to have larger type II fibers, but only 15 of these muscles had significant differences. Conversely, Howells et al (1978) reported that IIA and IIB fibers from the rat anterior tibialis had smaller myofibrils (.69 and .86μm^2 respectively) than from type I fibers (1.57μm^2). However, Tomanek et al (1973) observed that guinea pig ST muscles did not have clearly defined myofibrils but had some of the contractile material interrupted by spaces of cytoplasm. If this same phenomenon occurred in rat muscle (type I or ST) then Howells et al (1978) may have incorrectly calculated type I myofibrillar area. Myofibrils from guinea pig soleus (type I fibers) have smaller myofibrils than white vastus muscle (type II fibers) (Eisenberg et al, 1974; Eisenberg and Kuda, 1975). However, Stonnington and Engel (1973) found that the ratio of myofibrillar area to fiber area was the same in rat soleus and white vastus lateralis muscle at 83% and 85% respectively. Eisenberg and Kuda (1976) have reported the rat soleus

to be 100% type I fibers (SO) while the white vastus lateralis is 71% type II fibers (FTG). Type II fibers from rat tibialis anterior have a smaller percentage of myofibrillar packing density (59.3%) than IIA (60.3%) or type I (87.1%) fibers (Howells et al, 1978). However the authors assessed only a total of 20 fibers and used area measurements which do not correct for oblique sections. The myofibril volume density of human vastus lateralis was found to be similar in trained orienteers, untrained males and untrained females at 82.97%, 85.87% and 87.38% respectively (Hoppeler et al, 1973).

Fiber and myofibrillar areas were reported to increase 30 and 36% in triceps brachii after heavy resistance training, but myofilament packing densities did not change (MacDougall et al, 1976). More recently the authors reported that fiber areas increased significantly both in FT (39%) and ST (31%) fibers with heavy resistance training (MacDougall et al, 1979).

1.IG.2 M and Z Lines

Mouse EDL was reported to have thin Z lines and prominent M lines while the SOL had thick Z lines but no M line (S hafiq et al, 1969). FTG fibers from the guinea pig vastus lateralis had narrow Z lines (582-610Å), FOG and FTR fibers had an intermediate Z width (890Å) while the ST fibers of the soleus had wide Z lines (1100-1420Å) (Tomanek, 1973; Eisenberg et al, 1974; Eisenberg and Kuda, 1975; 1976). More recent measures from cat SOL (ST muscle), tibialis anterior (FOG) and caudifemoris (FG) have confirmed the result of the previous authors. Van Winkle and Schwartz (1978) observed that cat ST Z bands are wide (1250Å) while FOG and FG fibers are much narrower (875Å and 450Å respectively). However comparing properties from different muscles may not be valid.

From human muscle studies Cullen and Weightman (1975) reported that type I fibers did not differ significantly from Type II fibers according to Z line width. However the authors used only four subjects and took one biopsy from each non homogeneous muscle thereby obtaining various fiber types. The authors did not have a method to identify the fiber types they measured.

Payne et al (1975) identified significant differences between Z and M lines in fibers from both the human vastus lateralis and intercostal muscles. The authors observed that type I fibers had the widest Z line (94.7 nm), type IIB the smallest Z line width (68.7 nm) and type IIA had an intermediate Z line width (73.6 nm). The authors found the same trends with M line widths (i.e. type IIB fibers ahd a considerably narrower M line (59 nm) than that of either the type IIA (79.1 nm) or type I fiber (89.4 nm). The type IIA fiber can be seen to have intermediate structural characteristics, being closer to the type I fibers in M line width by the Z line width being closer to the IIB fiber.

Generally, both animal and human studies report the same results. Type I (or ST) fibers have wide Z and M lines, while type IIA (or FOG and FTR) fibers have narrow Z and wide M lines and type IIB (or FTW and FG) fibers have both narrow Z and M lines.

Eisenberg et al (1974) found that the measurement of Z width (and presumably also M width) is a subjective measure with measurement reproduction of only $\pm$50A. Z width also depends upon orientation, fixation and

contraction of tissue (which occurs in needle biopsies). The authors suggest using Z band volume as a measure.

1.IG.3 Mitochondria

Most studies indicate large differences in mitochondrial numbers and location between fiber types. Mitochondria from rat SOL muscle were reported to be the same size as in the vastus lateralis muscle despite the former being designated a FT muscle and the latter a ST muscle (Stonnington and Engel, 1973). However, Tomanek et al (1973) observed guinea pig FTW (vastus lateralis) to have few mitochondria which are round or oval, while FTR fibers from the same muscle have numerous mitochondria and are broad with a dense matrix and closely arranged cristae. ST fibers (SOL) had the most numerous and largest mitochondria with a lesser opaque matrix (Tomanek et al, 1973). The size of the longitudinal or transverse mitochondria was found to be the same in ST and FG fibers of the guinea pig but larger in FOG fibers (Eisenberg and Kuda, 1976).

Van Winkle and Schwartz (1978) indicated that cat SOL and tibialis anterior (ST and FOG fibers respectively) did not differ in mitochondrial number but were significantly greater than caudofemoralis(FG) mitochondria number. Mitochondria from rat and guinea pig FTG fibers orientated longitudinally are sparse (Stonnington and Engel, 1973; Eisenberg and Kuda, 1975; Tomanek et al, 1975; Eisenberg and Kuda, 1976) and arranged in longitudinal chains in the outer annulus. Mitochondria within FG fibers are usually found near the Z line. Mitochondrial volume was reported to be 2.2% (Stonnington and Engel, 1973).

Mitochondria of red guinea pig vastus (FOG) are found in high concentrations around the periphery and close to the nucleus and the neuro-

muscular junction (Eisenberg and Kuda, 1976). FOG fibers have greater mitochondria numbers than FTG fibers, but fewer than ST fibers (Stonnington and Engel, 1973; Tomanek et al, 1973; Eisenberg and Kuda, 1976).

The mitochondria of guinea pig SOL muscle was concentrated in the outer 1μ annulus of the fiber (Eisenberg et al, 1973). The total mitochondrial volume was 4.85% (Eisenberg et al, 1973). This compares to 7.4% from cross section and 5.7% from longitudinal sections of rat soleus muscle (Stonnington and Engel, 1973).

Similar to the animal research, human studies have shown that type I fibers have more and larger mitochondria than type II fibers (Ogata and Murata, 1969; Payne et al, 1975; Ingjer, 1979). However, Cullen and Weightman (1975) reported that type I and II fibers from deltoid, quadriceps, triceps and gastrocnemius had large overlapping ranges of mitochondria density, volume and number. It may be noted that all fiber types are able to increase their oxidative capacity with endurance training (Ingjer, 1979a; 1979).

Hoppeler et al (1973) have reported sex differences in mitochondrial volume densities. The authors found that untrained men had greater mitochondrial density than untrained women (5.19% and 4.08% respectively) (Hoppeler et al, 1973). In contrast, Bell et al (1979) have shown no sex differences in mitochondria of six year old children. The differences which Hoppeler et al (1973) observed may be explained by the relative activity of "untrained" men and women (i.e. "untrained" men are more active and will therefore have a greater mitochondrial density than "untrained" women), or this may reflect the muscle fiber composition for males and females.

Endurance trained athletes may have as high a mitochondrial volume density as 7.32% (Hoppeler et al, 1973), while heavy resistance training

may decrease mitochondrial volume density by as much as 26% and mitochondrial to myofibrillar volume by 25% (MacDougall et al, 1979). Endurance training increased the number of mitochondria in all fiber types from the vastus lateralis (Ingjer, 1979a; 1979), while heavy resistance training did not increase mitochondrial numbers in fibers from the triceps brachii (MacDougall et al, 1979). Without further mitochondrial increases to accompany fiber hypertrophy (which is associated with heavy resistance training), the mitochondrial volume density decreased (MacDougall et al, 1979).

1.IG.4 Lipid

Lipid droplets were rarely found in FTG fibers of the vastus lateralis of the guinea pig (Tomanek et al, 1973; Eisenberg and Kuda, 1975) and they were found to represent 0.012% of the total fiber volume (Eisenberg and Kuda, 1975). The lipid volume density of ST (guinea pig SOL) muscle is much higher at 0.2% and this was primarily distributed in the periphery of the cell (Tomanek et al, 1973; Eisenberg et al, 1974). FTR fibers (guinea pig vastus lateralis) also had high lipid inclusions (Tomanek et al, 1973).

Type I fibers in humans have numerous lipid droplets, while there is a medium lipid content in IIA fibers and few lipid droplets in IIB fibers (Ogata and Murata, 1969; Payne et al, 1975). Endurance trained orienteers were reported to have a higher lipid volume density (.85% of fiber volume) than either untrained men or women (.34% and .41% of fiber volume respectively) (Hoppeler et al, 1973). Trained men had a 2.5 fold greater lipid volume density than untrained men. Cullen and Weightman (1975) did not find differences in lipid contents from fibers of either human deltoid, quadriceps, triceps or gastrocnemius muscles. The latter authors did not have a method to determine the fiber type they had studied

ultrastructurally.

1.IG.5 Sarcoplasmic Reticulum (SR)

The SR in muscle forms a sleeve structure surrounding each of the myofibrils and has a high concentration of calcium ions. Upon a muscle action potential reaching the SR (via the transverse tubule network), the bound Ca^{2+} ions are released. The Ca^{2+} binds the troponin molecule on the actin filament which then allows interaction between the actin and myosin cross bridges. SR content appears to vary between animal fiber types. SR area was found to be significantly greater in FG muscles from the mouse, guinea pig, rabbit and cat than in ST muscles from each animal (Schafiq et al, 1969; Luff and Atwood, 1971; Stonnington and Engel, 1973; Tomanek et al, 1973; Eisenberg and Kuda, 1974; 1975; 1976; Heilmann et al, 1977; Van Winkle and Schwartz, 1978a). Longitudinal SR (LSR) volume percentages were reported to be significantly greater in FG fibers (caudfemoralis), followed by FOG (tibialis anterior) and ST (soleus) in the cat, although the terminal cisternae content is only significantly different when comparing FT to ST fibers (Van Winkle and Schwartz, 1978a).

Eisenberg and Kuda (1975) reported SR volumes of 4.59% in FTG fibers of the guinea pig. The guinea pig SOL muscles had been reported to have an SR volume of 2.9-3.5% of fiber volume (Luff and Atwood, 1971; Eisenberg et al, 1974). Surface areas of SR in the rat were found to be 1.83 and $3.82 \mu m^2/\mu m^3$ for slow and fast muscle respectively (Stonnington and Engel, 1973). These values are greater than that reported in the guinea pig for FTG ($1.32 \mu m^2/\mu m^3$) (Eisenberg and Kuda, 1975) and the ST ($0.9 \mu m^2/\mu m^3$) (Eisenberg et al, 1974).

There is a considerably greater T-system volume in FT fibers (vastus red and white) than in ST fibers (SOL) of the guinea pig. Thus, T-tubule volume density is greater in faster contracting fibers. However, the T-tubular surface area was reported to be similar in FTW, FTR and ST fibers (Eisenberg and Kuda, 1976). The latter authors did have a standard deviation of 30% of the mean, which does suggest a large variation. Luff and Atwood (1971) found that mouse T-tubules of FT fibers (EDL) possessed .4% of fiber volume while T-tubules of ST fibers (SOL) contained .22% of fiber volume. The surface area of SR in FT muscle was 3.1 cm^2/cm^2 while SR in ST muscle was 1.8 cm^2/cm^2. All the preceeding data compared SR in different muscles. A valid comparison of SR or any property should be made on different fiber types from the same muscle.

Unlike the animal studies several authors have found no difference in SR content among type I, IIA, IIB fibers of human vastus lateralis, external intercostal, deltoid, triceps or gastrocnemius muscles (Cullen and Weightman, 1975; Payne et al, 1975). Volume density and surface densities of SR were reported to be similar (3.78% and 1.694 m^2/cm^2 respectively) in the vastus lateralis of untrained men and women and trained orienteers who presumably had higher percentages of type I fibers (Hoppeler et al, 1973).

Eisenberg and Kuda (1975) observed two components of SR: (1) the terminal cisternae which contain granular material and are on each side of the T-tubule and (2) tubules with clear lumina that join one terminal cisternae to the next across either the A or I band region. These tubules run predominately longitudinally. Payne et al (1975) found sarcoplasmic reticulum in FTW fibers to be most extensive in the A band but they also had expansions into the I band, while Eisenberg and Kuda (1975) found the

opposite; SR was twice as abundant in the I band as in the A band. The previous authors both used guinea pigs for investigation of SR. The SR of FTR fibers in the guinea pig had a plexus in the A band and a less extensive I band network (Tomanek et al, 1973).

Several structural differences have been found in animal SR from fast and slow twitch muscles. Margreth et al (1972) observed electrophoretically separate protein patterns of SR between rat SOL and EDL muscle. The SOL had SR with several major bands with intermediate and fast mobilities and a decreased amount of material near the origin. EDL had one large band at the positive end.

Heavy and light SR vesicles have been identifed electrophoretically in SR of rabbit muscle. However quadriceps muscle (predominately the FT area) was centrifuged;therefore, it is unclear whether these different components of SR are from different fiber types or from variations within a fiber type (Meissner, 1975).

Tannic acid was used to study SR ultrastructure in rabbit skeletal muscle (Saito et al, 1979). The authors observed SR to have an assymetrical membrane of which 75% is composed of Ca^{2+} pump protein. The authors also observed heavy SR which has a large Ca^{2+} binding protein content and a light SR which has a lesser amount. The Ca^{2+} pump protein contains two specific Ca^{2+} binding sites and one ATPase binding site (Meissner, 1973). The previous author did not specify the location of rabbit muscle sampled nor did he determine the fiber type that was studied. It is therefore unclear whether the noted SR variations are due to different fiber types or variations within a fiber type. Saito et al (1979) hypothesized that heavy SR was derived from the terminal cisternae because similar fiber-like

characteristics were found in heavy SR and the terminal cisternae.

Earlier research by Heilmann et al (1977) reported rabbit FT muscle (psoas) had low and high density SR vesicles. The high density vesicles had Ca^{2+}, Mg^{2+} dependent ATPase activity while low density (light) vesicles had little activity. It is possible that the previous authors sampled an intermediate fiber type, especially when considering results from cat and guinea pig muscle, which showed that FT muscle had faster Ca^{2+} uptake and binding sites than the ST muscle (Fiehn and Peter, 1971; Van Winkle et al, 1978b). Sréter and Gergely (1964) reported that FT muscle (white vastus lateralis) from the rabbit had 9-15 times greater Ca^{2+} uptake than did the red semitendinosus. Various FT muscles from rabbits, cats and chickens showed greater rates and total uptake of Ca^{2+} than ST muscles from the same animals (Sréter and Gergely, 1964). Guinea pig FTW and FTR muscles were found to have identical calcium accumulating and calcium concentrating capacities, while ST muscles had considerably smaller maximal calcium uptake, but the same calcium concentration outside the SR after Ca^{2+} uptake (Fiehn and Peter, 1971). The slower rate of Ca^{2+} uptake by SR of ST muscle correlates with the prolonged relaxation time. The half relaxation time of guinea pig FTW, FTR and ST was 19.9, 18.5 and 113 ms (Fiehn and Peter, 1971). Huxley (1964) suggested that there is a possible relationship between the density of SR (and therefore Ca^{2+} release) and the muscle's speed of shortening. Greater SR volumes and capacity for Ca^{2+} storage and release might partly explain faster twitch times in FT or IIB fibers. M-ATPase activity has been shown to be greater in FT (EDL) than ST (SOL) muscle of the mouse (Bárány, 1967) which suggested a good correlation existed between twitch contraction time and M-ATPase activity. Edgerton and

Simpson (1969) argued that M-ATPase can only be a gross indicator of fiber contraction time since fibers with similar M-ATPase activity had varying contraction times. Recent fiber conversion studies by Heilman and Pette (1979) indicate that SR is important in twitch contraction time to peak tension and relaxation time although M-ATPase levels were not recorded.

1.IG.5a Summary of Sarcoplasmic Reticulum

SR appears to be greater in animal FT than ST muscles. SR was reported to have high volumes in both sarcomere A and I bands, while FTR fibers had extensive A band but little I bandnetwork. SR of FT fibers were thought to have high density vesicles which have a fast Ca^{2+} uptake while ST fibers had low density vesicles and a lower rate of Ca^{2+} uptake. Human SR is thought to follow the same trend as animal SR, however the literature is sparse.

1.IG.6 Myofilaments

The contractile protein filaments of skeletal muscle are actin and myosin. Myosin from FT muscle was found to be structurally different from ST muscle (Lowey and Risby, 1971; Sreter et al, 1972; Arndt and Pepe, 1975). Both actin and myosin are directly involved in contraction.

A third type of filament has been observed in frog cardiac and skeletal muscle and rabbit psoas muscle (Maruyama et al, 1977; de Remedios and Gilmor, 1978). This material comprises 5% of the fibrillar protein and is thought to be connectin and elastic protein (de Remedios and Gilmor, 1978). These filaments appear in the I and H band and especially in the Z disc. They are approximately the same size as actin filaments (2-7 nm). This filament was thought to join the Z discs (de Remedios and Gilmor, 1978). There is no evidence to suggest that this third filament is involved in the

contraction process.

The measure of "myofilament packing density" involves obtaining the number of thick myofilaments (myosin) per unit area (Brandt et al, 1967). Actin filaments are not used because of the erroneous calculations that would result from contracted muscle (i.e. from a needle biopsy).

Myofilament packing density was reported to decrease (112.9/.5μ^2 post training; 119.2-128/.5μ^2 area pre-training) following sprint training (running up bleachers) with a concomitant increase in distance between myosin (503Å post training; 478-492Å pre-training) (Penman, 1969). Strength training was found to increase packing density in the quadriceps by Penman (1970), while MacDougall et al (1976) found myofilament packing density of the triceps brachii did not change with strength training. Brandt et al (1967) studied the relationship between myofilament packing density and sarcomere length in frog semitendinosus muscle. They found that packing density is directly proportional to sarcomere length (i.e. the distance between adjacent myofilaments is an inverse square root function of the sarcomere length). A slightly longer sarcomere length recorded by Penman (1970) in post training samples (1.99μ compared to 1.7-1.82μ at pre-training) may therefore account for his seemingly erroneous conclusion that strength training decreases packing density. While the myofilament packing density may not change with strength training there is an increased myofibril size and area and thus an increase of the total number of myofilaments (MacDougall et al, 1976; 1979). This total increase in myofilaments could account for an increase contractile force or tension output in strength trained muscle. An increase in myosin filament packing density would decrease the cross bridge length. This would assume that the angle of cross bridge attachment did not change.

Summary

Biochemical and histochemical studies have shown that type I fibers have high oxidative enzyme and low glycolytic enzyme activity, while type II fibers are low in oxidative enzymes and high in glycolytic enzymes (Brooke and Kaiser, 1970; Engel, 1974; Askanas and Engel, 1975; Essen et al, 1975). Type I fibers have a strong acid reaction to myosin ATPase and a weak alkaline myosin ATPase reaction. The reverse is true in type II fibers. Type IIA, B and C fibers can usually be differentiated at three pH levels of preincubation for myosin ATPase. Type IIA has a stronger oxidative reaction than IIB, but is weaker than type I. Type IIA has no reaction at pH 4.6 for ATPase, while IIB and IIC have strong reactions (Brooke and Kaiser, 1970). At a pH of 4.3 type IIA and IIB are inhibited and IIC will give a moderate stain for myosin ATPase (Brooke and Kaiser, 1970).

Correlation between physiological and histochemical profiles indicate that the type I fiber corresponds to the slow twitch fatigue resistant fiber. Type IIA fibers appear to correspond to fast twitch fatigue resistant fibers. If a stimulus is maintained for a sufficient time, fatigue will occur in type IIA fibers. This is unlike the type I fiber which appears not to fatigue despite 60 minutes of continuous interval tetani (Burke et al, 1971; Burke and Tsairis, 1974; Garnett et al, 1979). The fast twitch fatiguing fibers correspond to type IIB fibers. The IIB fiber also appears to produce the greatest tension (Barany and Close, 1971; Burke et al, 1971; Burke and Tsairis, 1974; Tesch and Karlsson, 1978) and have the greatest rate of tension development (Gonyea and Bonde-Petersen, 1977).

In males, type II fibers are larger than type I fibers but this is not the case in females (Brooke and Engel, 1969; Polgar et al, 1973; MacDougall, 1979).

Type I fibers have been shown to have three times the triglyceride content (Tomanek et al, 1973; Essen et al, 1975; Payne et al, 1975) more and larger mitochondria (Ogata and Murata, 1969; Payne et al, 1975; Ingjer, 1979; MacDougall et al, 1979), and more myoglobin (Alway et al, 1980) than type II fibers. No difference was found between fiber types in SR content (Hoppler et al, 1973; Cullen and Weightman, 1975; Payne et al, 1975). The myofilament packing density for each fiber type is unknown. Packing densities have not been found to change with training (MacDougall et al, 1976).

The physiological, histochemical and ultrastructural correlation of fiber types have given greater insight into muscle mechanisms and function of different fiber types, but there is still much that is not known.

1. II. Myoglobin

The presence of myoglobin in skeletal muscle has long been known. There are little or no myoglobin differences within the same general family of animals (i.e. fox vs. dog; zebra vs. horse), but there are differences in muscle myoglobin levels between different species of animals (Dorbe and Lehman, 1976).

Red muscle fibers are rich in oxidative enzymes, mitochondria and myoglobin and are thus well endowed for oxidative energy production. However, they can still contract anaerobically either by alactic or lactic acid pathways (Close, 1972). In contrast, white fibers are generally larger

in diameter and are rich in phosphorylase and depend mostly on the Embden-Meyerhoff pathway for contractile energy. The myoglobin and mitochondrial content in these fibers are lower than in red fibers (Close, 1972).

1. IIA Myoglobin Changes

1. IIA.1 Altitude:

Myoblogin content can be affected by altitude. Gimenz et al (1977) exposed dogs to simulated altitude and found that muscle myoglobin concentration increased from 3.67±1.07 to 5.47±.9 mg/g fresh muscle. Skeletal myoglobin was found to be greater in men adapted to living at altitude 4,400 m than in persons living at 50 m (Reynafarge, 1962). Moreover several authors have shown that rats and guinea pigs when exposed to altitude (12,500-14,900 ft.) for 75 days, increased their skeletal muscle myoglobin 70% above sea level values (Vaughan and Pace, 1956; Tappan and Reynafarje, 1957).

1. IIA.2 Development of Myoglobin

Human fetal skeletal muscle myoglobin is less than 1% of adult levels despite low fetal arterial PO_2 levels (Longo et al, 1973). Myoglobin therefore appears to be synthesized after birth and is not important in the facilitation of oxygen diffusion or as an oxygen store in the fetal stage (Longo et al, 1973). Biorch (1949) and Kagen and Christian (1966) have reported that human cardiac myoglobin concentration is greater than skeletal myoglobin at birth, but the relationship is reversed between 1-2 years of age.

1. IIA.3 Seasonal Changes

Seasonal changes for myoglobin content have been found in species such as the red backed vole and the snow shoe hare (Rosenmann and Morrison,

1965; Morrison et al, 1966). The colder months had an associated increase in myoglobin and the reverse was true for the warmer summer months for these animals. It would appear that these animals lower their respiration rate to such a degree that the oxygen saturation of the blood decreases, and thus the muscles receive less oxygen. To compensate for this, more myoglobin is produced in order to increase the oxygen supply from the blood to the muscle.

1.IIA.4 Changes with Physical Activity

Myoglobin content also changes with physical activity patterns. The sharp increases in skeletal muscle myoglobin after birth and smaller increases in cardiac myoglobin parallels the increased exercise load on these muscles (Longo et al, 1973). Myoglobin was shown to increase in rat skeletal muscle following moderate running exercise over 17 days, while short intense exercise did not affect myoglobin content (Lawrie, 1973). The author was not able to differentiate between fiber types, only whole muscle groups. In a similar study, rats subjected to vigorous treadmill running for 15 weeks had a higher capacity for running and an 80% increase in myoglobin content of the quadriceps and hamstring muscles than control groups (Pattengale and Holloszy, 1967). The authors concluded that the increased myoglobin content accounted for the greater running capacity. However they did not consider the effect of increased mitochondrial oxidative enzymes or the number of capillaries around each fiber which might have increased with endurance training (Ingjer, 1979a; 1979). This would allow greater amounts of oxygen to be available and ultimately used in the mitochondria. If this occurred, the oxygen carrying role of myoglobin would be implemented, thus making the endurance trained muscle,

with greater myoglobin levels, a more efficient muscle for oxygen utilization.

Although physical activity levels appear to play an important role in determining the concentration of myoglobin in normal skeletal muscle (Pattengale and Holloszy, 1967), myoglobin levels of dystrophic muscle are not affected by exercise (Booth, 1978). The latter author found that after 45 days of treadmill running normal hamsters increased gastrocnemius myoglobin significantly ($p < .005$) while there was no change in dystrophic animals.

1.IIB Roles of Myoglobin

In 1939, Millikan described three basic roles of myoglobin or what he termed muscle hemoglobin:

1. Myoglobin acts as a transporter of oxygen from the blood capillary system to the mitochondrial enzymes within the cell.
2. Myoglobin may act as a catalyst with oxidative enzymes.
3. Myoglobin may also act as a storage for oxygen.

1.IIB.1 Myoglobin as a Transporter

The oxygen uptake of muscle under steady state was studied during which time the storage function of myoglobin by being static, disappears, thus revealing the kinetic function of myoglobin in oxygen transport (Wittenberg et al, 1975). The authors found that myoglobin serves to transport oxygen to the mitochondria within the blood physiological PO_2 range of 0-100 mm Hg.

The lower limits of myoglobin adaptation to hypoxia was studied using carboxymyoglobin measures (Coburn and Mayers, 1971). The authors determined oxygen tension for myoglobin of dog hamstring muscle at normal

arterial PO_2 (67-120 mm Hg) to be 4.7 mm Hg. The mean oxygen-myoglobin tension decreased 1.5 mm Hg as arterial PO_2 fell from 490-40 mm Hg. When arterial PO_2 was lower than 35-40 mm Hg, the mean oxygen-myoglobin tension decreased to 0.5-1.5 mm Hg. The authors hypothesized that arterial PO_2 below 20-25 mm Hg represent the lower limits of myoglobin effectiveness in hypoxic muscle.

1.IIB.2 Myoglobin: Storage of Oxygen

Myoglobin has only limited capacities for storage of oxygen. Millikan (1939) estimated that the body's myoglobin store would be used in seven seconds of "extreme activity".

The muscles of diving mammals or birds have a much larger myoglobin content than terrestrial mammals (Wittenberg, 1970). During a dive, the blood supply to the muscles is cut off thus relying on stored oxygen (Irving, 1964).

Millikan (1939) showed that muscle oxygen demand rises instantaneously with the onset of contraction and oxygen supply may be limited as the contracting muscle occludes the blood vessel. Myoglobin acts as an oxygen buffer supplying oxygen to the mitochondria during contraction and taking up oxygen from the capillary blood during relaxation (Millikan, 1939; Wittenberg *et al*, 1975).

1.IIB.3 Evidence for Myoglobin as a Catalyst

Gloster and Harris (1977) suggested a new role for myoglobin. Based on radioactive assays of labelled oleic acid, they observed that myoglobin bound fatty acid to low molecular weight proteins in rat cardiac muscle. They then investigated red and white muscle. In red muscle they observed a small peak of radioactivity corresponding to low molecular weight

fat binding. No radioactive peaks were found in white muscle. Adding horse myoglobin gave a greater radioactive reaction. These results give some evidence that myoglobin may also function as a transport protein for fatty acids in the cytoplasm. This fits well with Millikan's (1939) hypothesis that one of the roles of myoglobin was to act as a "catalyst" for oxidative enzymes. This fat transportation theory by Gloster and Harris (1977) would support the findings that myoglobin rich red skeletal and myocardial muscle fibers have a high capacity for oxidation and ulilization of free fatty acids (Opie, 1969; Close, 1972).

1.IIC Localization of Myoglobin

Until recently the association between fiber type and myoglobin content was unclear. James (1968) used the benzidine peroxidase reaction to demonstrate myoglobin rich and myoglobin poor fibers in transverse sections of animal muscle. He also showed differences in muscle spindles. Nuclear bag fibers from "red" muscle had high myoglobin activity and contracted slowly while nuclear chain fibers from "white" muscle have less myoglobin and contract rapidly.

1.IIC.1 Myoglobin and Its Relationship to Fiber Type

Morita et al (1969) observed fibers that stained darkly in myoglobin were also high in oxidative enzymes (NADH-TR reaction) while the reverse occurred in low myoglobin fibers. Alway et al (1980) found strong correlation between the M-ATPase reaction and myoglobin reaction of human vastus lateralis muscle. They observed that type I fibers had a high intensity of myoglobin activity while type IIA showed intermediate activity and IIB a light myoglobin reaction.

1.IIC.2 Regions of Myoglobin Localization Within a Fiber

Fibers stained for myoglobin localization using various benzidine peroxidase reactions (after glutaraldehyde fixation) show uniformly stained transverse sections (James, 1968; Morita et al, 1969; Alway et al, (1980) indicating that myoglobin is uniformly distributed within the muscle fiber. Kagen and Gurevich (1967) using a fluorescent-antibody technique reported that myoglobin in human skeletal muscle was confined to alternate cross striations, although they were unable to indicate fluorescence to be in the A or I band. Subsarcolemmal regions were clearly fluorescent. This suggested that myoglobin in muscle was immobile, although staining of the Z lines and sarcolemmal regions suggests possible artifacts. The foregoing study relied on a formaldehyde fixation which has been shown to destroy muscle myoglobin (James, 1968). There is no membrane in a myofibril which could confine a soluble protein like myoglobin to a particular region in the sarcomere (Wittenberg, 1970). Therefore for myoglobin to be localized it must be bound to another structural protein. However the myoglobin does not appear to follow exclusively either myosin or actin filaments. The fixation medium used by Kagen and Gurevich (1967) may have caused myoglobin to localize in sites of precipitation. Myoglobin is probably not bound to myosin, and other suggestions that it is, may be due to artifact (Wittenberg, 1970).

1.IIC.3 Myoglobin and Activity Patterns

Rapidly contracting muscles that are activated several hundred times per second (e.g. bat cricoid and the sound producing fish bladder) lack or have only minimal traces of myoglobin (Wittenberg et al, 1970). Muscles which have slow repetitive activity have very high myoglobin levels

(Wittenberg et al, 1970), and recent evidence by Gloster and Harris (1977) suggested that myoglobin is associated with metabolic properties attributed to type I fibers.

There is a parallel between myoglobin content and the activity pattern and metabolism of muscle. Myoglobin was observed to be high in: slowly contracting (ST) muscle (Wittenberg et al, 1970), fibers that utilize lipid as a fuel source (Gloster and Harris, 1977) and fibers which require high oxygen levels for oxidation of fuel to ATP (Wittenberg et al, 1970; 1975).

Type I fibers have all the mentioned profiles and therefore it would be expected that myoglobin would be highest in type I fibers, lowest in IIB and intermediate in IIA based on each fiber's physiological and metabolic profile.

1. III. Evidence for Fiber Type Conversion

Conversion of fibers from type I to type II or the reverse, have been studied in both animals and humans. It is impossible for individual muscle fibers to be studied during pre and post experimental manipulation. Thus whole muscles or the percentages of fibers within a muscle were studied with regard to possible fiber changes.

1.IIIA Fiber Changes from Cross-reinnervation

Important information has been obtained from cross-reinnervation studies where the nerve that normally innervated a fast muscle was crossed over so that it innervated a slow muscle and vice versa (Buller et al, 1960). Such cross-reinnervation studies in animals have shown histochemical conversions of fiber types (Romanul and Van Der Muelen, 1966; 1967; Dubowitz, 1967).

Buller et al (1969) found that a fast muscle which was re-innervated with a slow nerve had the M-ATPase activity (alkaline pre-incubation) reduced. The re-innervated FT muscle assumed the biochemical and histochemical properties of the nerve (ST) that had innervated that muscle. These results were confirmed in other research (Romanual and Van Der Meulen, 1966; 1967; Dubowitz, 1967; Samaha et al, 1970). Isometric twitch responses also reversed with cross-reinnervation (Buller et al, 1960). It was apparent that the nerve which innervated a muscle determined the biochemical, histochemical and contractile properties of that muscle; however, the nature of the neural influence was not explained.

Salmons and Vrbova (1977) hypothesized that the total impulse activity determined the speed of muscle contraction and the production of tension. This was not found by Pette et al (1973) who observed that both low and high frequencies of continuous stimulation resulted in greater increases in twitch tension and tetanic tension than continuous stimulation; however, the contraction times were similar to that of the previous authors. Thus it appears that total impulse activity is not the most important factor affecting changes in contractile properties.

1. IIIB Electrical Tetanization and Fiber Changes

Tetanization a slow muscle was found to result in an increase of speed of contraction (Vrbova, 1969). However, Buller and Lewis (1965) found that tetanizating a muscle did not totally convert twitch responses, tetanic responses and tetanus to twitch ratios and tension development from slow to fast characteristics. Tetanizating a muscle does not appear to cause total fiber conversion.

1.IIIC Mechanisms of Conversion

1.IIIC.1 Physiological Changes

Salmons and Sréter (1976) attempted to determine whether the mechanism for fiber conversion resulted from the type of neural traffic to the muscle. The authors cross-innervated the rabbit soleus muscle with a fast muscle. One group had electrodes implanted into the cross-innervated muscles to produce slow interval stimulation. The right leg for all animals acted as a control. They observed that the cross-reinnervated muscle with the electrode, possessed the same contractile, ATPase and slow light myosin chains as the control (unoperated) muscles. The cross-innervated muscles which were not stimulated by an electrode changed their twitch characteristics, ATPase activity and structurally possessed fast myosin chains. In the same study, fast EDL muscle implanted with slow stimulus electrodes, achieved contraction and ATPase activity similar to the soleus muscle. This was confirmed by Heilmann and Pette (1979). The half relaxation time of EDL muscle also increased after stimulation (Heilmann and Pette, 1979). The latter authors observed decreased Ca^{2+} dependent ATPase and decreased Ca^{2+} transport in EDL, which paralleled a progressive decrease in Ca^{2+} dependent and a Ca^{2+}, Mg^{2+} dependent phosphoprotein formation (Ca^{2+} pump protein) and reduced rate of dephosphorylation. The electrophoretic polypeptide and phosphoprotein patterns of EDL changed to the patterns reported by Heilmann *et al* (1977) for slow twitch muscle (Heilmann and Pette, 1979). This transformation paralleled a rapid increase in time to peak tension during initial stimulation and increased more slowly during stimulation over 14-125 days (Heilmann and Pette, 1979). The authors noted contraction changes occurred

before conversion of the light myosin chain was observed. The correspondence in time of SR and twitch contraction characteristics suggest that SR may determine the contractile properties (i.e. twitch reponses) in fast and slow twitch muscle (Heilmann and Pette, 1979).

1.IIIC.2 Myosin Chain Alterations

Electrophoresis studies have shown that the fast myosin light chains were completely replaced by slow myosin light chains in fast twitch muscle subjected to cross-innervation or chronic stimulation (Salmons and Sréter, 1976; Pette and Schwez, 1977). Furthermore such FT muscle was found to increase its capacity for aerobic metabolism as demonstrated by oxidative enzyme reactions (Pette et al, 1975; Romanul and Van Der Meulen, 1966; 1967), and increase its mitochondrial level to that of the ST soleus muscle (Salmons et al, 1978).

1.IIIC.3 Ultrastructural Changes

Z band width from chronically stimulated rabbit FT muscle was found to increase. Z bands from EDL and tibialis anterior muscle increased from a normal width (40 nm) to a wide Z band (79 nm) equal to that of the control ST soleus muscle (80 nm) (Salmons et al, 1978).

1.IIIC.4 Fiber Conversions with changed Neural Impulse Activity

Salmons and Sréter (1976) rule out the possibility that the physiological and biochemical differences between fast and slow muscle are attributable to differences in the trophic influences of their motor nerve. The authors based their conclusions on observations that: (1) in the absence of an actual change in the pattern of impulse activity reaching a muscle, cross-reinnervation has no significant effect; and (2) a change

in the pattern of impulse activity unaccompanied by a change in the motor innervation can produce effects equalling or surpassing those brought about by cross-reinnervation.

1. IIID Fiber Changes with Electrical Stimulation

Munsat et al (1976) showed significant fiber type changes in five subjects, of which four underwent electrical stimulation of the rectus femoris, while one subject was given an electrical stimulation to cause tenatomization of the same muscle. Muscle biopsies were taken before and after slow electrical stimulation. The four subjects showed significant increases in percentages of type I fibers. Both I and II fiber types increased in size ($p < .001$). The tenotomized muscle showed type I fibers to decrease from 40.2% to 25.4% and their mean diameter also decreased ($p < .001$). A certain degree of caution is necessary when interpretating the results of Munsat et al (1976). First, the differences in location of the second biopsy sample (post stimulation) may account for the different fiber distribution that was observed. Another consideration is the possibility that the observed fiber shift does not represent a conversion from type II to type I but rather a selective atrophy or dropout of type II fibers. This would give the illusion of increases in type I fibers and fiber conversions. However, this seems to be disproved by the fact that all fibers increased in area. It may be possible to convert human type II to type I fibers or conversely given the correct stimulus.

1. IIIE Fiber Conversions with Training

Such conversion of fiber types in man as noted above, have not been observed in either endurance training (Gollnick, 1973; Saltin, 1976; Jansson and Kaiser, 1977) or heavy resistance training (Thorstensson et al, 1977;

MacDougall et al, 1979). However endurance training has been observed to decrease the type IIB to IIA ratio (Jansson and Kaiser, 1977). Biopsies were taken from the vastus lateralis, gastrocnemius and detoid muscles before and after training. No conversion from type II to I was found, but IIA, (a more oxidative fiber than IIB) increased significantly ($p < .01$) compared to type IIB. Some subjects had a complete conversion of all IIB fibers. A rather highly oxidative IIC fiber was seen in greater predominance in orienteers as compared to controls (Jansson and Kaiser, 1977). Type IIC fibers are usually seen to represent a very small percentage of the total fiber population and have been assumed to be undifferentiated or intermediate fibers (Kugelberg, 1977). Jansson et al (1978) reported evidence to suggest fiber conversion from type I to IIC after interval running (90-100% VO_2 max.). After sprint training all subjects had increased type IIC fibers and decreased the percentage of type I fibers compared to endurance training (distance running). After sprint training, three of the four subjects had a higher percentage type IIA and IIB fibers and an increased IIB/IIA ratio. The authors concluded that endurance training caused a conversion from IIC to type I fibers and sprint training caused the reverse to be true. This is supported by a continuity in the M-ATPase stain intensity within the type IIC fiber population from fibers staining similar to type I fibers to fibers staining similar to IIA fibers. To explain the finding that the percentage of type IIC fibers increased after sprint training but not after endurance training Jansson et al (1978) have hypothesized that the transformation process between type IIC and IIA is slower than between type I and IIC. Unlike preceeding studies (Gollnick, 1972; Saltin, 1976; Jansson, 1977), the work done by Jansson et al (1978) gives evidence that fiber conversion may occur with physical activity.

1.IIIF Fiber Changes with Inactivity

Muscle inactivity by immobilization has failed to show evidence of fiber conversions (MacDougall et al, 1976; Sargeant et al, 1977; Houston et al, 1979). Sargeant et al (1977) observed that after leg immobilization fiber cross sectional areas were reduced 42%. Both type I and II fibers were affected being reduced from 3410 to 1840μm^2 and from 3810 to 2390μm^2 in cross sectional area respectively. In six of seven subjects, type I fibers showed a greater atrophy than type II fibers, being 46% reduced in type I fibers and 37% in type II fibers. In a more recent study, Houston et al (1979) observed marathoners who had been training for at least four years. The subjects had their leg immobilized with a cast for seven days, then allowed normal walking activities for eight days. Following the 15 days of detraining, 15 days of training (running) occurred and the subjects attempted to achieve their pre-detraining distances as soon as possible. The authors observed that there were no significant changes in fiber type composition. However, this experiment lasted only 15 days and may be too short to observe any conversions, if indeed they do occur. There is no evidence for fiber conversion (based on myosin ATPase reactions) of immobilized healthy (no neuromuscular disease) muscle.

Reported differences in fiber type percentages for athletes of various sports (Edstrom and Ekblom, 1972; Costill et al, 1976; Jansson and Kaiser, 1977) may be more related to genetic selection than fiber conversion. Although the possibility of conversion cannot be entirely ruled out, especially with the recent work by Jansson et al (1978).

Summary

The present investigation was designed to increase the current knowledge regarding the ultrastructural characteristics of specific skeletal muscle fiber types.

Previous studies have used longitudinal fiber typing by M and Z lines but this method is not possible for cross sectional identification and needle biopsies because of contracture. Cross sectional fiber typing has been reported using mitochondrial densities and volumes but has not proven to be a reliable method because of overlapping ranges between fiber types. Freezing the tissue for cryostat sectioning for M-ATPase identification, before fixation for electron microscopy, results in distortion of the fine structure of the tissue. The present study used a myoglobin staining technique which identified fiber types for ultrastructural analysis. This technique has been shown to be an accurate method for distinguishing fiber types with minimal freezing artifact.

Most studies have used area or content measures for morphological investigation. However, volume measures have been shown to be a more accurate measure, and remains valid when sections are not cut at exactly 90°. This study used stereological analysis to obtain normative data for different fiber types.

Previously reported animal research has shown greater sarcoplasmic reticulum development in fast twitch muscle. This has not been found to occur in human muscle. However, such results may have been due to faulty fiber typing. This study included sarcoplasmic volume density measurements in fibers which were known to be either type I or type II.

Myofilament packing densities have been included in some studies, but without the knowledge of the fiber being studied. In this present study the myofilament packing density of known fiber types was measured for the first time. Ultrastructural mitochondrial analysis of the mitochondrial content in human muscle fibers has yielded similar results to animal research. Mitochondrial volume density was studied as confirmation of previous research. Lipid measures were also made to confirm earlier longitudinal investigation and obtain normative volume density data for each fiber type.

In summary, various stereological measures were selected to study ultrastructural characteristics of fiber types.

3. Definition of Terms

For the purpose of this thesis the following definition of terms shall apply:

A. Enzymes

Alpha glycerophosphate dehydrogenase (αGPD); the enzyme on the outer mitochondrial membrane which facilitates the oxidation of extramitochondrial NADH. αGPD is considered to represent glycolytic activity within a fiber.

Creatine phosphokinase (CPK); the enzyme responsible for adding a phosphate compound to creatine, to form a high energy compound, creatine phosphate.

Lactate dehydrogenase (LDH); the enzyme which converts lactate (or lactic acid) to pyruvate in the Emden-Myerhoff pathway.

Myokinase (MK); the enzyme which facilitates the action of hexokinase in catalyzing the reaction between glucose or fructose and adenosine

triphosphate by which glucose 6-phosphate or fructose 6-phosphate and adenosine diphosphate are formed.

Myosin adenosine triphosphatase (M-ATPase); the isolated myosin enzyme which catalyzes the hydrolysis of adenosine triphosphate to supply the necessary energy for muscular contraction.

Nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR); an oxidative enzyme of the citric acid cycle which is found in high levels in fibers which have a high capacity for oxidative energy production.

Phosphofructokinase (PFK); the enzyme which catalyzes the biochemical conversion of fructose 6-phosphate to fructose 1,6 diphosphate.

Succinate dehydrogenase (SDH): the oxidative enzyme which oxidizes succinate to fumarate.

B. Components of Muscle

Longitudinal sarcoplasmic reticulum (LSR); sarcoplasmic reticulum volumes which are calculated from longitudinal muscle preparations.

Magnesium ions (Mg^{2+}); ions from the inorganic metal magnesium which act as a co-enzyme and assist in binding ATP to an enzyme site (e.g. ATPase).

Sarcoplasmic reticulum (SR); the network of longitudinally orientated cisterna which run parallel to the contractile filaments within a sarcomere.

C. Fiber types

Fast twitch fibers (FT); fibers with a short contraction ($\lesssim$60 ms) and relaxation.

Fast twitch glycolytic fibers (FTG); fibers which have the characteristics of FT fibers and have high glycolytic enzyme activity.

Fast twitch oxidative glycolytic fibers (FOG); fibers which have characteristics of both FTG and SO fibers, being fast contracting and relaxing and having both high glycolytic and oxidative enzyme activity.

Fast twitch red fibers (FTG); fast twitch fibers which were obtained from muscles predominately red in color (i.e. high myoglobin levels).

Fast twitch white fibers (FTW); fast twitch fibers which were obtained from muscles predominately white in color (i.e. low myoglobin levels).

Slow twitch fibers (ST); fibers with long contraction ($>$60 ms) and relaxation times.

Slow twitch oxidative fibers (SO); fibers which have the characteristics of ST fibers and have high oxidative enzyme activities.

Type I fibers; fibers which have an acid stable myosin ATPase (i.e. M-ATPase is inactivated at a preincubation pH of 10.0).

Type IIA fibers; fibers which have basic stable M-ATPase but have the ATPase enzyme inactivated at a preincubation pH of 4.6.

Type IIB fibers; fibers which have basic stable M-ATPase (i.e. M-ATPase is inactivated at a preincubation pH of 4.3 and 4.6).

Type IIC fibers; fibers which have both acid stable and basic stable M-ATPase (i.e. M-ATPase is active at preincubation pH's of 10.0, 4.6 and 4.3).

D. Motor Units

Continuously firing long interval motor units (c.l.m.u.s.); motor units which can be driven continuously and attain regular firing intervals at rates as low as 10/s.

Fast contracting fast fatiguing motor units (FF); motor units which have FT characteristics and fatigue the quickest of the three motor unit types.

Fast contracting fatigue resistant motor units (FR); motor units which have FT characteristics but take much longer to fatigue than FF units.

Intermittently firing short motor units (i.s.m.u.s.); motor units which could not be driven continuously and did not fire below 20/s.

Slow contracting motor units (S); motor units which have ST characteristics.

E. Physiological Characteristics

Tension; the quantity of physical force exerted by a muscle or group of muscles against an object.

Tetanic tension; tension which a muscle or group of muscles produce during the state of complete tetanus.

Twitch; a brief phasic contraction of a muscle fiber or the short sudden contractile response of a motor unit to a single maximal stimulus.

Twitch tension; the quantity of force exerted from a single fiber or motor unit during a contraction.

F. Muscles

Anterior latissimus dorsi (ALD): the anterior section of the large upper back muscles of mammals, birds and rodents which is considered a "red" muscle (i.e. high in myoglobin).

Extensor digitorum longus (EDL); a FT muscle of the lower leg of rodents and mammals which lies immediately lateral to the tibialis anterior muscle.

Posterior latissmus dorsi (PLD); the posterior sections of the upper back muscles of most mammals, bird and rodents which is considered a "white" muscle (i.e. low in myoglobin).

Soleus (SOL); a highly oxidative, ST msucle found below the gastrocnemius msucle on the lower limbs of most rodents or mammals.

G. Measurement/Analysis

Electromyography; a standard method for recording electrical patterns of muscular activity.

Freezing artifact; any distortion of a muscle fiber which is due to freezing of the muscle sample prior to fixation (e.g. swelling or breakings in the cell wall; ruptured mitochondria).

Morphometric analysis; a method introduced by Weibel (1972) for calculation of ultrastructural components of muscle fibers.

H. Morphometric Volume Measures

Mitochondrial volume density (Vv_{mit}); the ratio of 168 test points which fall on the mitochondrion to those falling elsewhere on the fiber.

Myofibril volume density (Vv_{myof}); the ratio of 168 test points which fall on myofibrills to those falling elsewhere on the fiber.

Cytoplasm volume density (Vv_{cyto}); the ratio of 168 test points which fall on cytoplasm to those falling elsewhere on the fiber.

Sarcoplasmic reticulum volume density (Vv_{sr}); the ratio of 168 test points which fall on cytoplasm to those falling elsewhere on the fiber.

Lipid volume density (Vv_{lip}); the ratio of 168 test points which fall on cytoplasm to those falling elsewhere on the fiber.

Myofilament packing density; the number of myosin filaments per square one-half micron.

CHAPTER 2

Methods

2.I Subjects

Five male subjects aged 20-21 years were selected randomly from a list of 31 volunteers. All subjects were healthy and active, but were not trained for a specific sport or activity. The subjects were second year physical education students at McMaster University. These were assumed to represent a normal population of young active males. The biopsy procedure was explained to each subject, whereafter they all gave written consent.

2.II Biopsy Procedure

Tissue samples were taken from the right vastus lateralis of each subject. Selection of this muscle allowed comparison with many previous clinical and research studies. Moreover, since the vastus lateralis contains both type I and II fibers (in more or less equal proportion) one is able to compare both fiber types within the same muscle (as opposed to comparing fibers from two different muscles).

The needle biopsy technique as outlined by Bergström (1962) was modified by the addition of a suction tube and syringe. This suction allowed larger biopsy specimens of approximately 70 mg. Two samples were excised from a single incision. One sample was prepared for routine histochemistry, while the other sample was prepared for electron microscopy using standard fixation procedures.

2.IIIA Histochemical Preparation

The excised muscle sample was mounted cross sectionally on a cork block and frozen in isopentane suspended in liquid nitrogen. Serial sections were cut on a cryostat (AO cryo-cut microtome) at -22 °C. The sections were processed for histochemical ATPase reactions at preincubation pH's of 10.0, 4.6 and 4.3 for classification of type I, IIA, or IIB fibers (Brooke and Kaiser, 1970). The interpretation of the ATPase reaction is summarized in table 2.1A.

Table 2.1A - ATPase Reaction

Preincubation pH	Fiber Types			
	I	IIA	IIB	IIC
10.0	weak	strong	strong	strong
4.6	strong	weak	intermediate	intermediate
4.3	strong	weak	weak	strong/intermediate

An NADH-TR reaction was also run on several sections to determine the oxidative enzyme activity of the fibers (Novkoff et al, 1961). The interpretation of this reaction is summarized in table 1B.

Table 2.1B - NADH-TR Reaction

	Fiber Types			
	I	IIA	IIB	IIC
Enzyme Activity	strong	intermediate	weak	intermediate

2.IIIB Preparation of Sample for Ultrastructural Analysis

2.IIIB.1 Staining for Myoglobin:

The second sample was taken through the same incision from the biopsy site and immediately placed in 2% glutaraldehyde in .1 M sodium cacodylate buffer, then stored in the buffer for an additional 24 hours to ensure all the glutaraldehyde was removed. The sample was removed from the buffer, orientated cross sectionally on a cork block as for cryostat sections and then frozen in isopentane suspended in liquid nitrogen. It was our experience that freezing tissue after fixation, caused minimal artifact. However, freezing the sample prior to fixation caused considerable damage.

Sections 30μ thick were first cut on the cryostat and then floated onto a diaminobenzidine-hydrogen peroxide solution for 30 minutes at 37°C. For further details see Appendix I. It has been previously shown that this is a valid technique for identifying fiber types in fixed tissue (Alway et al, 1980). This technique identified each fiber type, based upon their myoglobin content. Histochemically, the peroxidase activity of myoglobin of tissue fixed in glutaraldehyde converts diaminobenzidine in the presence of hydrogen peroxide to the primary reaction product benzidine blue (James, 1968). Non-enzyme oxidation of the primary reaction product forms benzidine brown which is the final reaction product seen under the microscope (James, 1968). The myoglobin interpretation of intensity reactions are summarized in Table 2.2.

Table 2.2 - Myoglobin Reaction

	Fiber Types		
	I	IIA	IIB
Reaction Intensity	strong	intermediate	weak

2.IIIB.2 Validation of Technique

An initial study showed that the diaminobenzidine-peroxidase reaction identified skeletal fiber types accurately (Alway et al, 1980). Biopsied muscle was obtained from the right vastus lateralis of three male subjects (aged 21-22 years) and histochemical methods outlined in Section IIIa were used to determine fiber types from serial cyostat section. One of the sections was fixed in 2% glutaraldehyde for four hours at 0°C, then washed in sodium cacodylate to remove the fixative and finally frozen as for histochemical procedures. The tissue sample was cut on a cryostat at 15μm and attached to glass slides containing a potato paste adhesive. The slides were placed in a diaminobenzidine-hydrogen peroxide solution to stain for myoglobin intensity (see Appendix 1).

Light microscope photographs were taken from the same field of fibers in both histochemical and myoglobin reactions. From 842 fibers examined, 99.1% of those classified as type I by the M-ATPase reaction stained darkly for myoglobin, 94.8% of those classified as type IIB stained lightly and 80.3% of those classified as type IIA stained intermediately.

The oxidative enzyme activity matched the myoglobin reaction. The strongest oxidative enzyme activity was found in fibers with the strongest myoglobin reaction. The reverse was found in fibers which showed the weakest myoglobin reaction, while fibers with intermediate oxidative enzyme activity also had an intermediate myoglobin reaction.

Plate 1. LIGHT MICROGRAPH OF FIXED MYOGLOBIN STAINED MUSCLE SECTIONED BY A MICROTOME

1. Muscle from vastus lateralis.

2. Section is approximately 2.0 μm in thickness.

3. Type I fibers have stained darkly for myoglobin.

4. Type II fibers have stained weakly for myoglobin.

5. Magnification is approximately X 250.

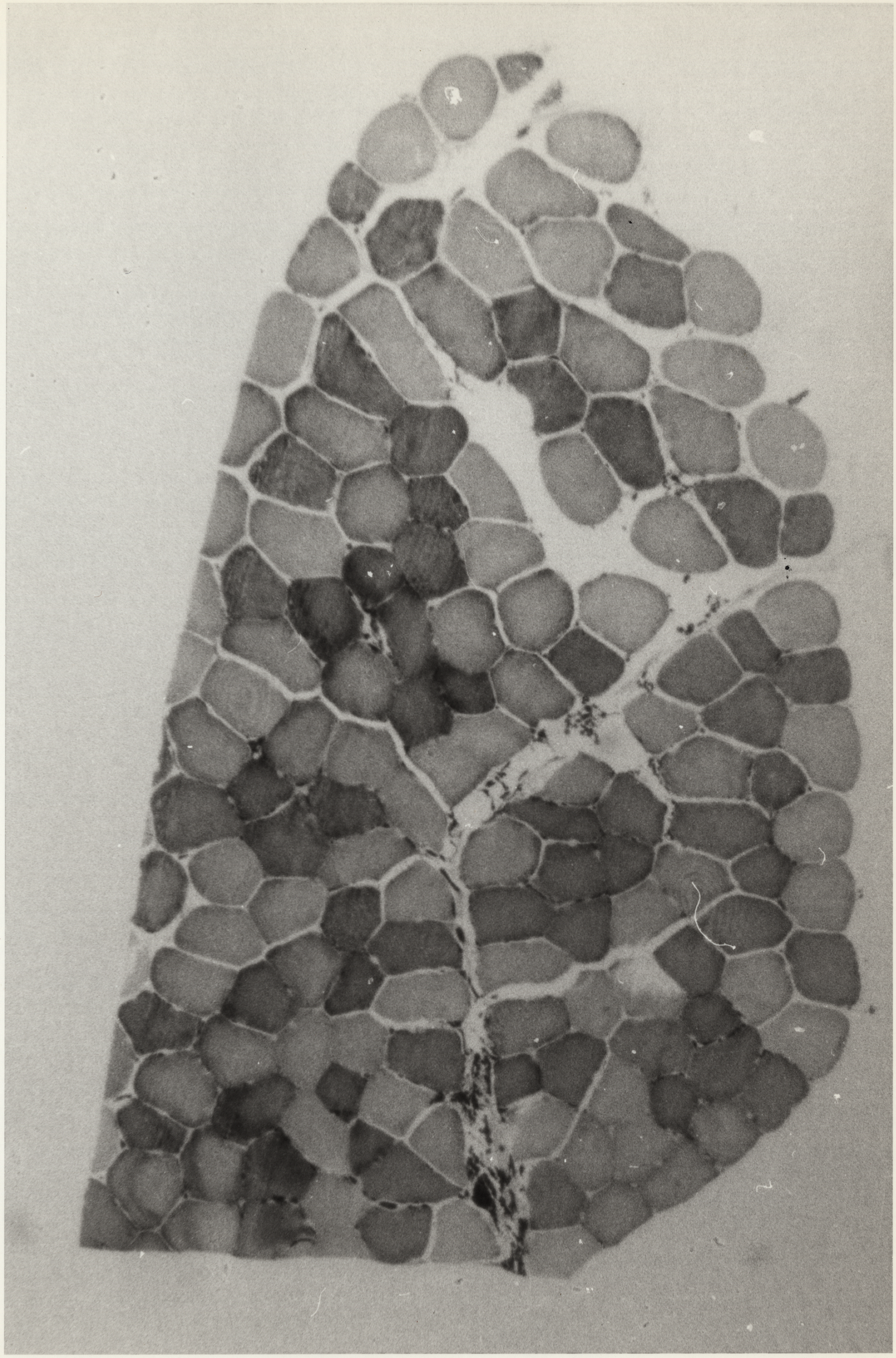


PLATE 1

It was concluded that the myoglobin reaction was a valid technique for identifying fiber types in glutaraldehyde fixed tissue.

2.IIIB.3 Electron Microscope Preparation

The stained skeletal muscle sections were post-fixed in 1% osmium tetroxide in .1 M sodium cacodylate for two hours at 5°C. The sections were rinsed in water for ten minutes then stained with uranyl acetate for one hour at 21°C. The sections were rinsed with water one final time then dehydrated with increasing strengths of ethanol alcohol and propylene oxide. The sections were infiltrated with spurr-resin, embedded into capsules then allowed to polymerize at 60°C.

The spurr blocks containing the tissue sample were sectioned on a microtome by an electron microscope technician. 2.0 μm sections were cut and mounted on glass microscope slides. The slides were photographed through a light microscope at a magnification of 40X and printed to 8½ x 11 inches giving a final magnification of approximately 250X. Ultrathin sections of 0.5 μm were cut from the same spurr blocks and mounted on 3.05 mm, G400HH Copper/Rhodian Gilder Grids then stained with 0.1% Reynold's lead citrate. The ultrathin sections were then viewed through a Phillips 300 Electron Microscope (EM) to ensure they were in true cross section. Any sections which showed the banding effect of repeating sarcomeres were rejected. In instances where the section was judged to be too oblique, the spurr block was realigned and new sections cut.

2.IV Fiber Type Identification

The photographs of the myoglobin stained fibers were compared to the ultrathin section of the same fiber at scan magnification on the EM. Each fiber was individually identified from the light microscope photograph

Plate 2. IDENTIFICATION OF FIBERS FOR ELECTRON MICROSCOPY

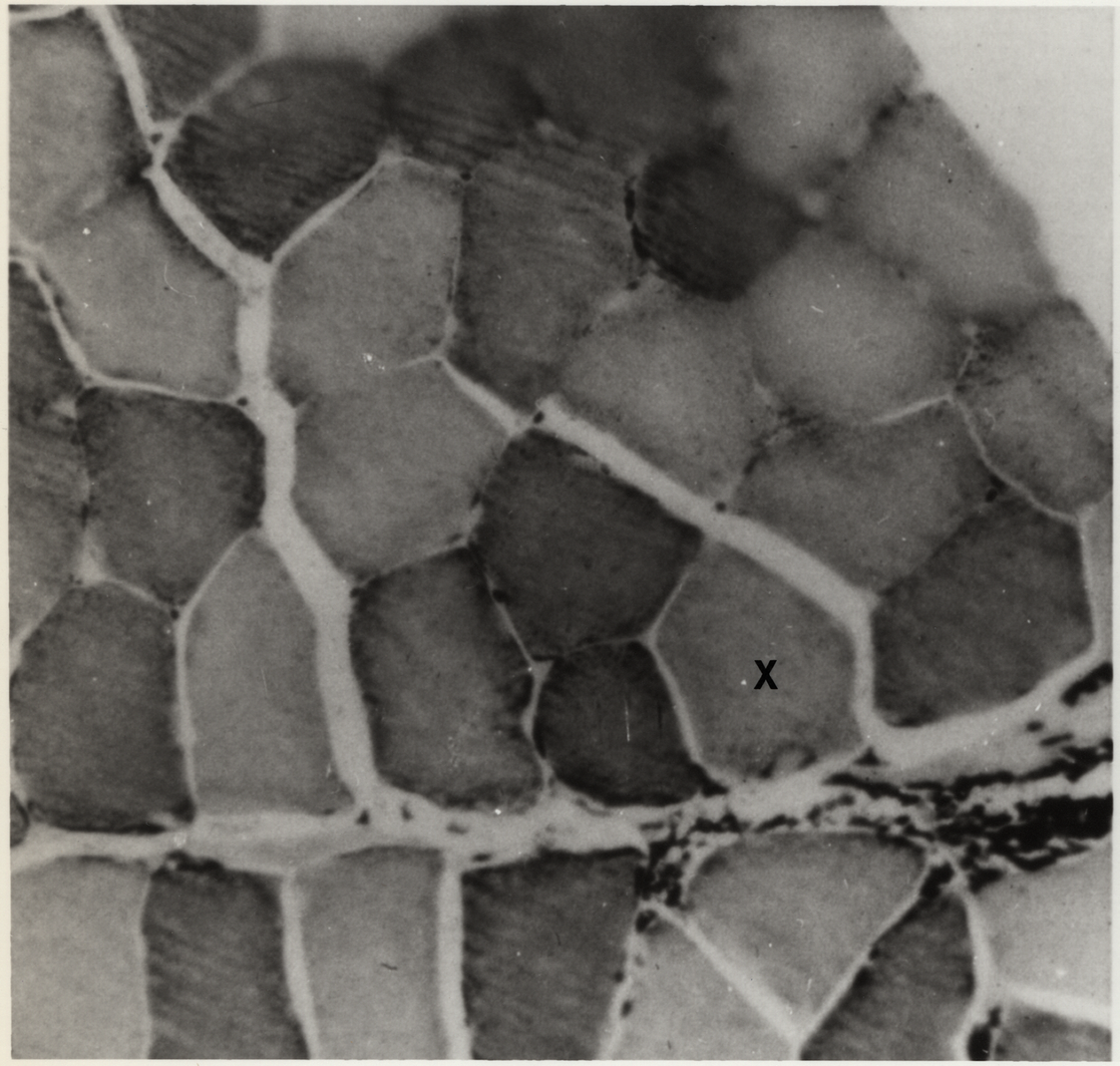
1. Comparison of light microscope photograph of EM thick section to the same fibers from the ultrathin section taken by the Electron Microscope.

2. X marks the same fiber in both photographs.

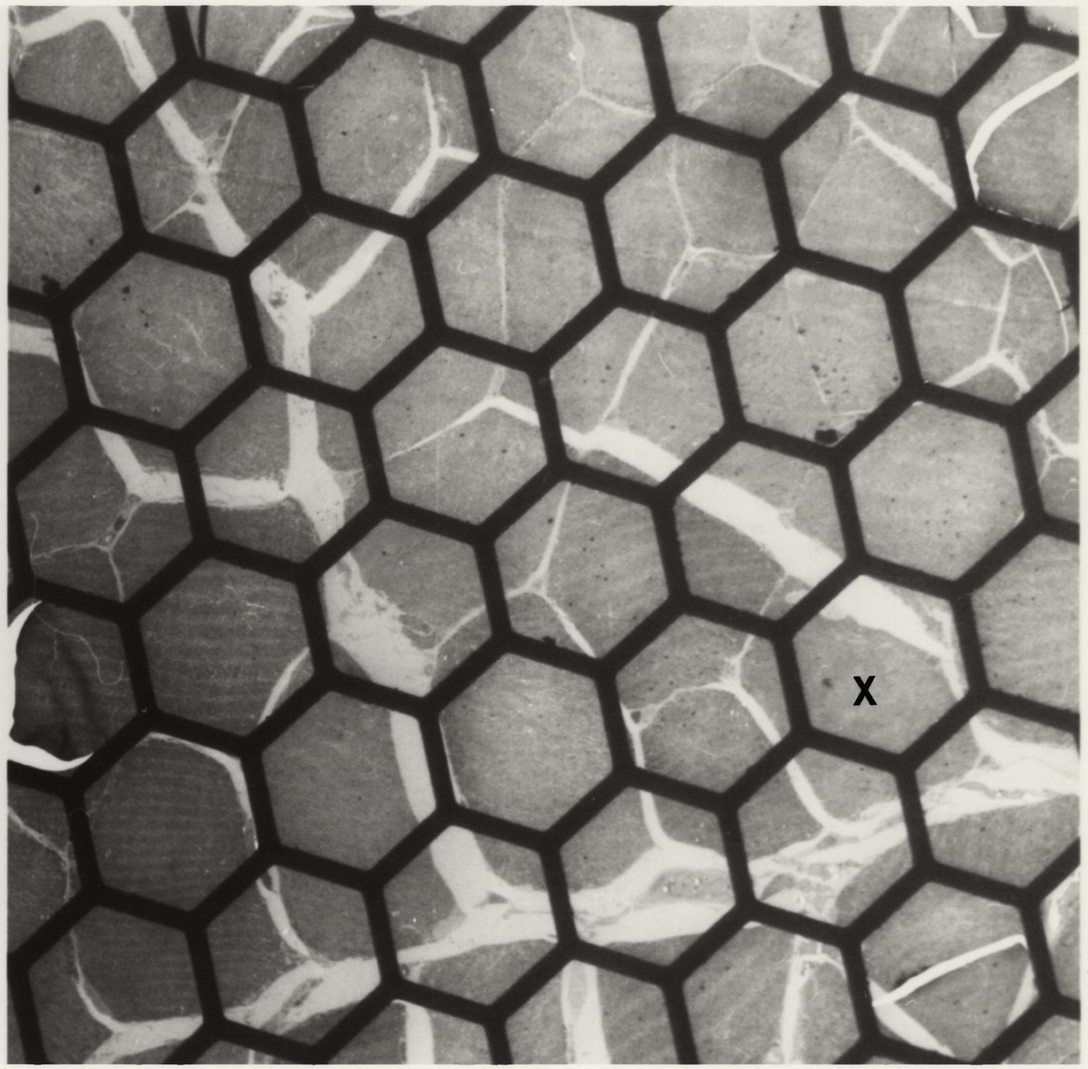
3. Magnification is approximately X 350.

PLATE 2

IDENTIFICATION OF FIBERS FOR ELECTRON MICROSCOPY



Light Microscope



Electron Microscope

and located at scan magnification on the EM viewing screen. The fiber was then given a number which was recorded directly onto the light microscope photograph to ensure that the fiber was only selected once. Although the dark staining fibers were easily identified, it was found that the relatively thin sections when viewed through the light microscope would not always permit positive identification of IIA vs IIB fibers. Consequently, for the purpose of this present study it was decided to identify fibers as either type I or II.

Fifty fibers of each type were randomly selected from each biopsy sample. Three random non-overlapping micrographs per fiber were taken at a magnification setting of 11 (X 7260) on the Philips 300 electron microscope. To ensure that the magnification remained constant, a measurement grid with 2160 lines/mm was photographed on each film of approximately 30 micrographs at the same magnification (X 7260).

2.V Ultrastructural Analysis

Identification of various ultrastructural components and particularly the SR network were difficult when the negative electron micrographs were viewed. As a result, positive contact prints were made by means of a specially constructed apparatus which permitted prints of the whole role. The positive 35 mm film was viewed using a Recordax MPE-1 Film Reader which projected the micrographs onto a 18" x 18" viewing grid. This gave the film a final magnification of approximately 140,000 X on the viewing screen.

The stereological 168 point counting technique was used to determine the volume densities of ultrastructural components (Weibel, 1973). In all instances stereological measurements were made "blind"; i.e. without the knowledge of which fiber type was being studied. It has been shown

that this method of morphometric analysis is still accurate with ultrathin sections cut at oblique angles up to 70° (Weibel, 1973). However, cuts less than this angle will cause an underestimation of various cellular components (Weibel, 1973). Cross sectional analysis of components by area measurement is inaccurate with any sectioning angle other than 90°.

Stereological equations were used to relate the flat profiles seen in sections to the three-dimensional structures from which they came. For example, volume density is a three-dimensional property that can be estimated by counting the fraction of 168 test points that falls on the two-dimensional profile of a structure. Specifically, the volume density of the sarcoplasmic reticulum (Vv_{sr}) in the fiber (Vf) is estimated by the ratio of test points falling on the sarcoplasmic reticulum (P_{sr}) to those falling elsewhere on the fiber (Pf), giving $Vv_{sr}/Vf = P_{sr}/Pf$. The standard equations are given with the assumption that the structures are randomly dispersed in space (Weibel, 1973). In this manner, volume densities for myofibrils (Vv_{myof}) mitochondria (Vv_{mit}), cytoplasm (Vv_{cyt}), sarcoplasmic reticulum (Vv_{sr}) and lipids (Vv_{lip}) were determined. Similarly, the volume density of these components could be expressed in terms of the myofibrillar volume density. For example, the ratio of sarcoplasmic reticulum volume to myofibril volume could be expressed as Vv_{sr}/Vv_{myof}. Only interior cell measures were made for each measure. The volume measurements which were obtained are summarized in Table 2.3.

Plate 3. ELECTRONMICROGRAPH OF CONSTITUENTS WHICH WERE STUDIED

1. Vastus Lateralis Muscle
2. a) MYOF - Myofibrillar protein

   b) MITO - Mitochondria

   c) CYTO - Cytoplasm

   d) SR - Sarcoplasmic Reticulum
3. Magnification X 7260

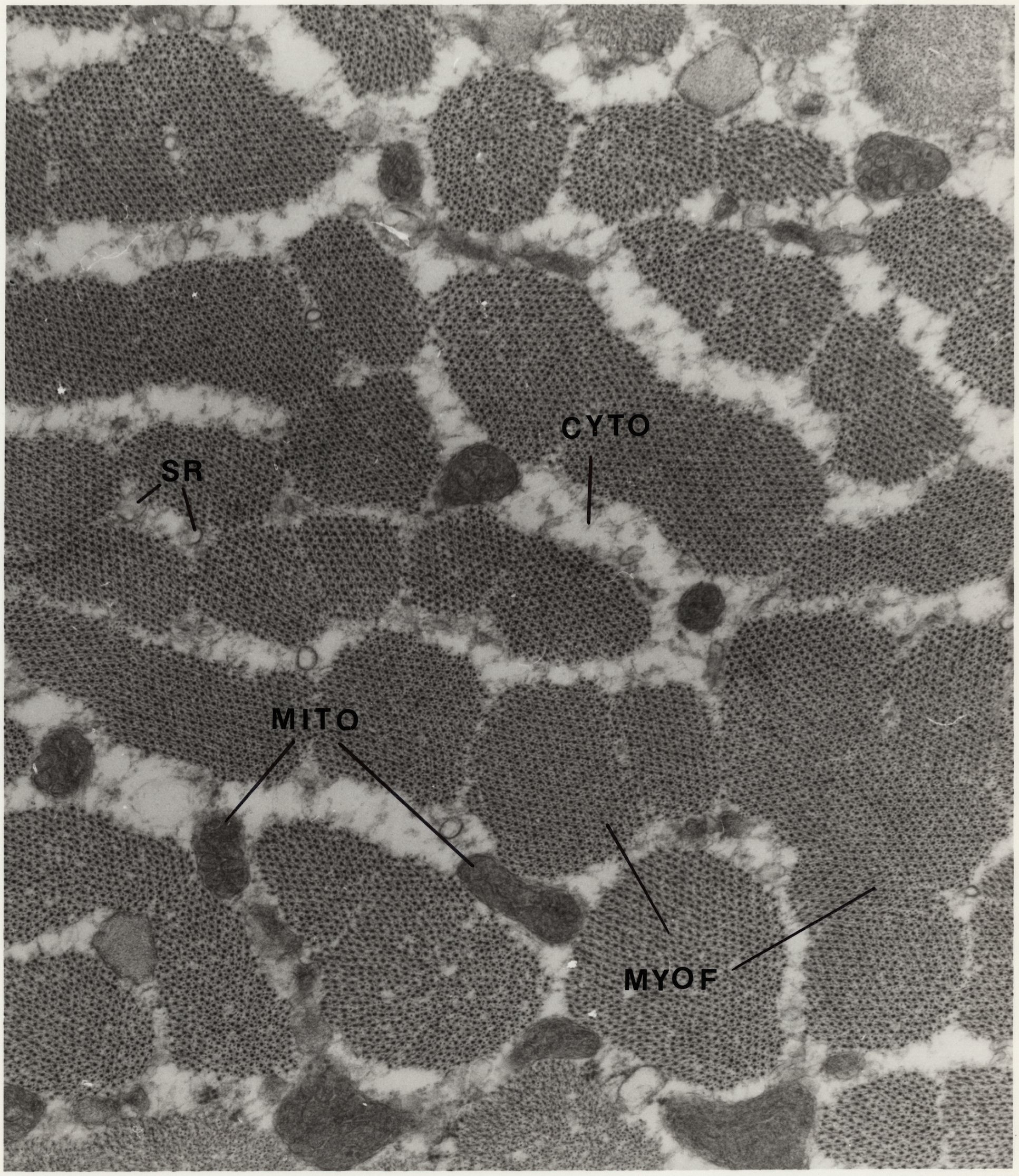


PLATE 3 Electron Micrograph of Constituents which were Studied

Plate 4. ELECTRONMICROGRAPH SHOWING LIPID DROPLETS

1. Vastus lateralis muscle

2. LIP - Lipid droplets

3. Magnification X 7260

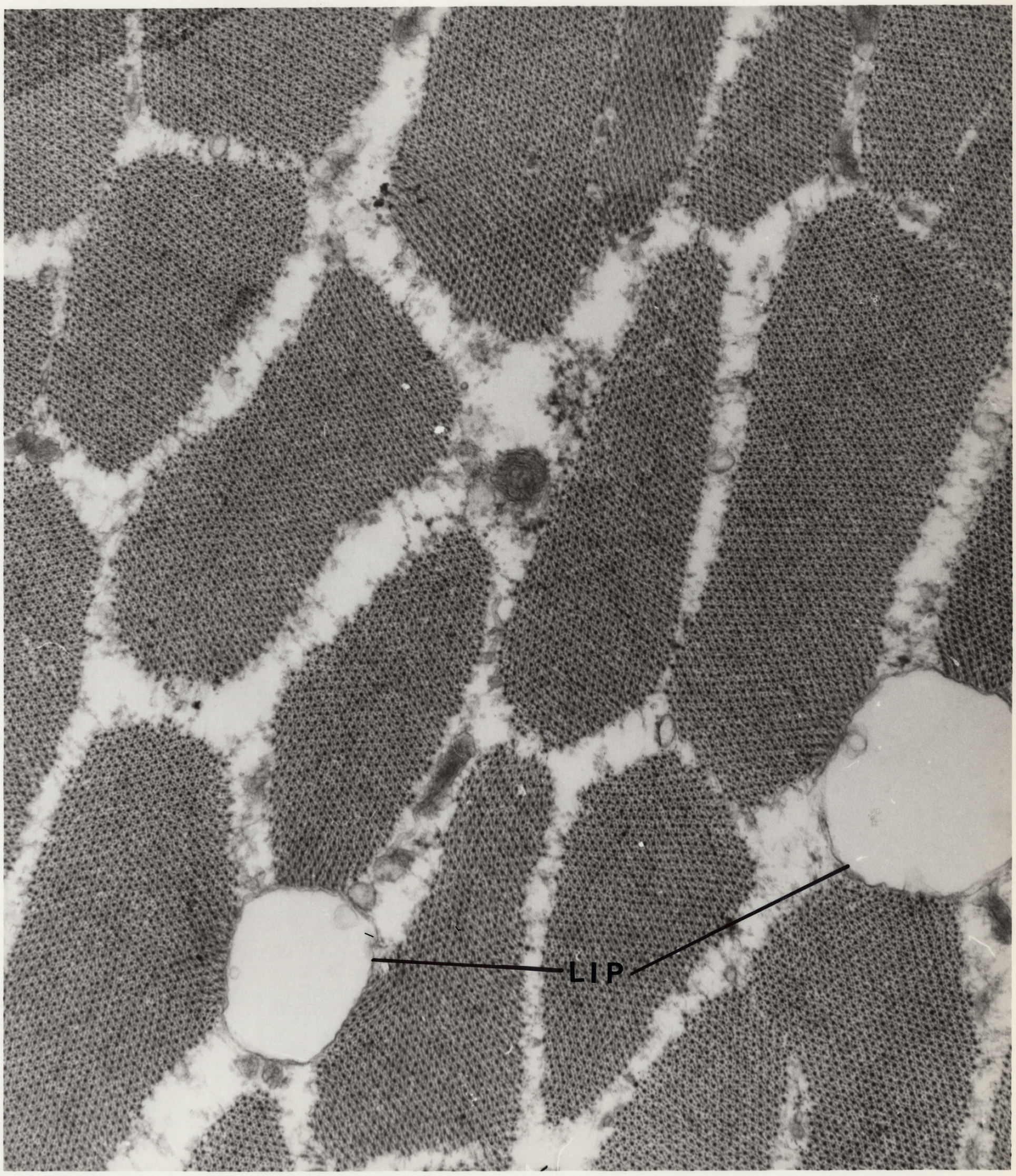


PLATE 4 Electron Micrograph showing Lipid Droplets

Table 2.3 - Volume Measures

	Components				
	Myofibril	Mitochondria	Cytoplasm	SR	Lipid
Measures	Vv_{myof}/Vf	Vv_{mit}/Vf	Vv_{cyt}/Vf	Vv_{sr}/Vf	Vv_{lip}/Vf
		Vv_{mit}/Vv_{myof}		Vv_{sr}/Vv_{myof}	

These measures were selected for two reasons: (1) to provide maximal information concerning the ultrastructural components of the fiber types; and (2) to compare the results obtained from the measures of this study to previous research which used similar measures on unclassified fiber types.

2.VI Myofilament Packing Density

The myofilament packing density for each fiber type was determined from the same projected magnification as the previous volume measures on those fibers which were considered to be in true cross section. One area was randomly selected from each micrograph for the calculation of packing density. The number of myosin filaments which fell within a 5" x 5" grid was then counted. This value was then extrapolated to the number of myosin filaments per one-half square micron ($.5\mu^2$). Thirty type I and II fibers each, were investigated in this manner for every subject.

This measure was selected to add to the present knowledge of muscle structure and function. Previous studies have not been able to determine which fiber type was studied. This measure was also useful in obtaining indirect evidence of crossbridge configurations between fiber types.

2.VII Fiber Areas

Cross sectional areas from 50 type I and 50 type II fibers were calculated by means of a 5 mm^2 counting method (Edström and Tolegard, 1969) from 8½" x 11" photographs (magnification X330) of the myoglobin thick sections cut from the resin blocks. The fiber areas were determined from the EM prepared tissue and not from histochemically stained tissue. This was felt to represent a better area measurement for comparison to the ultrastructural variables since the treatment of all EM tissue was identical. This would eliminate area differences between EM and histochemical samples otherwised caused by shrinkage or swelling.

2.VIII Statistical Procedures

A Student's independent two tailed t test was used for each subject to determine if the means of the organelles examined in type I fibers were significantly different from that found in type II fibers.

The means of each subject's organelle data was then pooled into one group. The mean and standard deviation of the pooled subject data were determined. A Student's independent two tailed t test was conducted between the pooled means of type I and II fibers for each organelle to determine statistical significance.

CHAPTER 3

Results

3.I Mean Myofibrillar Volume Density

Vv_{myof} for each fiber type are illustrated for each subject in Figure 3.1 (p. 75) and in Appendix III. The combined data is given in Figure 3.0 (p. 74).

The combined means for Vv_{myof} of type I and II fibers were 80.73 ($\pm$1.54)% and 82.48 ($\pm$1.31)% respectively. This difference is significant ($p < .02$). Individually, each subject except TC showed type II fibers to have significantly greater Vv_{myof}. Type II Vv_{myof} had 1.75% greater total cell volume than type I Vv_{myof}. There was little inter-subject variance for both type I and II fibers as shown by a small coefficient of variance (1.9 and 1.6 respectively). Coefficient of variance is the quotient of the standard deviation divided by the mean.

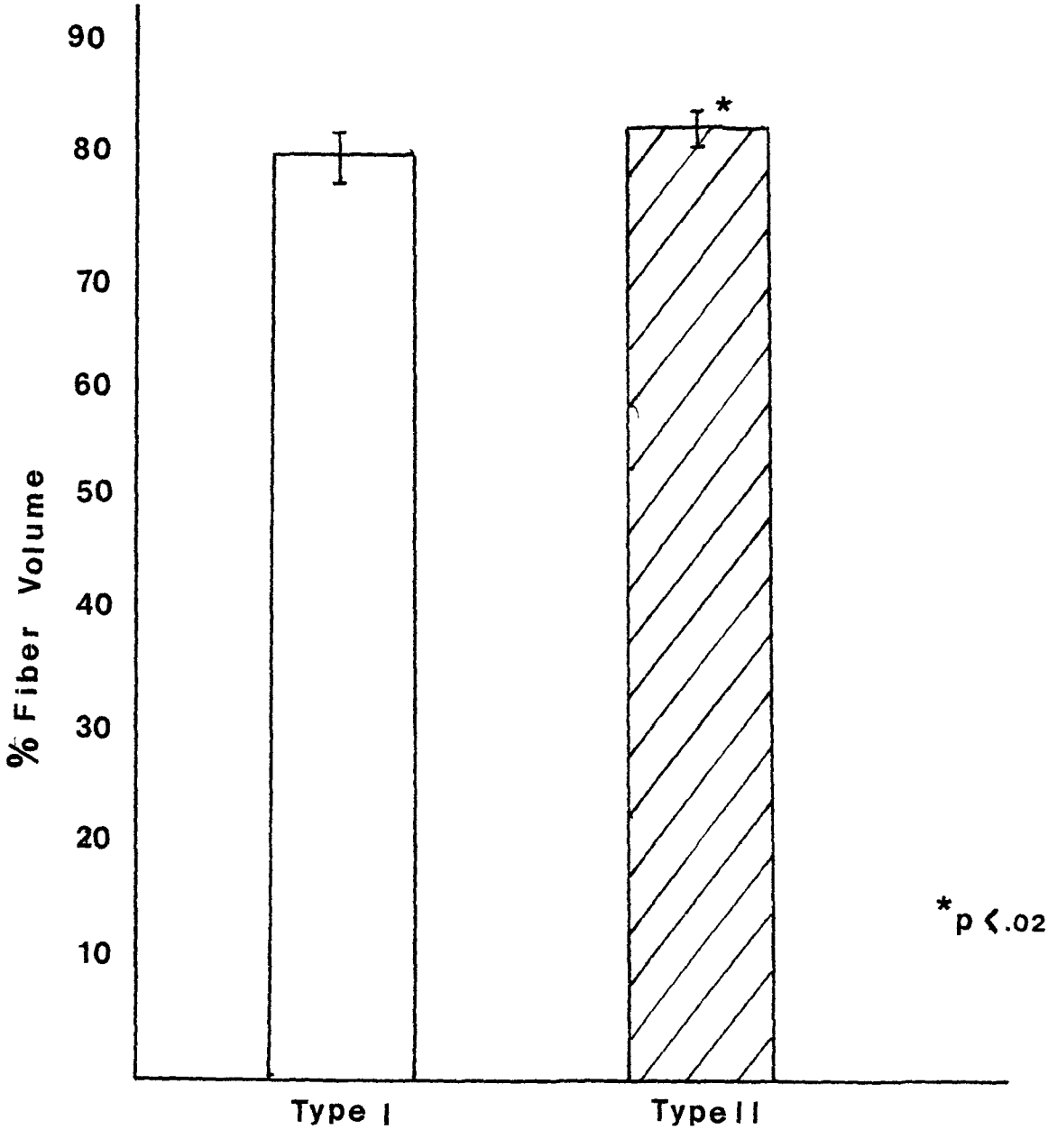
3.II Mitochondrial Volume Density

Vv_{mit} measures are presented for each subject in Figure 3.3 (p. 78) and in Appendix IV. The grouped measures are illustrated in Figure 3.2 (p. 76).

Vv_{mit} in type I fibers was significantly greater than in type II fibers ($p < .001$). Type I fiber had 5.78 ($\pm$.978)% and type II had 1.43 ($\pm$.508)% of the fiber volume represented as mitochondria.

Subject GB and TC possessed the lowest type I Vv_{mit} at 4.52 ($\pm$3.38)% and 5.01 ($\pm$3.41)% respectively which was significantly less ($p < .01$) than the other three subjects. (Coefficient of variance for type I fibers was 16.9). There was also significant inter subject Vv_{mit} variances within

Figure 3.0 MYOFIBRILLAR VOLUME DENSITY($\bar{X} \pm SD$) WHEN SUBJECTS WERE CONSIDERED AS A SINGLE GROUP



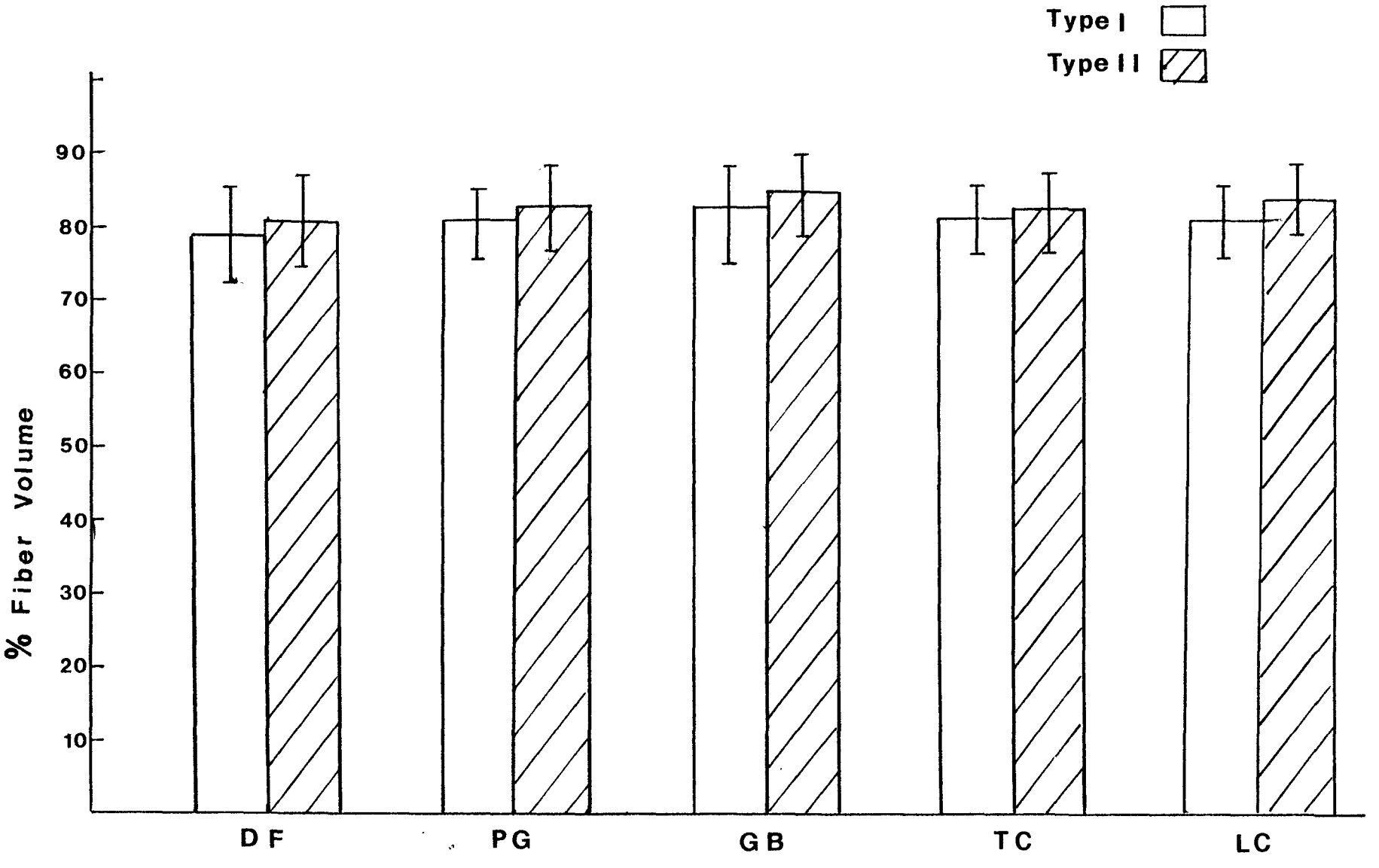


Figure 3.1 MEAN MYOFIBRILLAR VOLUME DENSITY FOR EACH SUBJECT

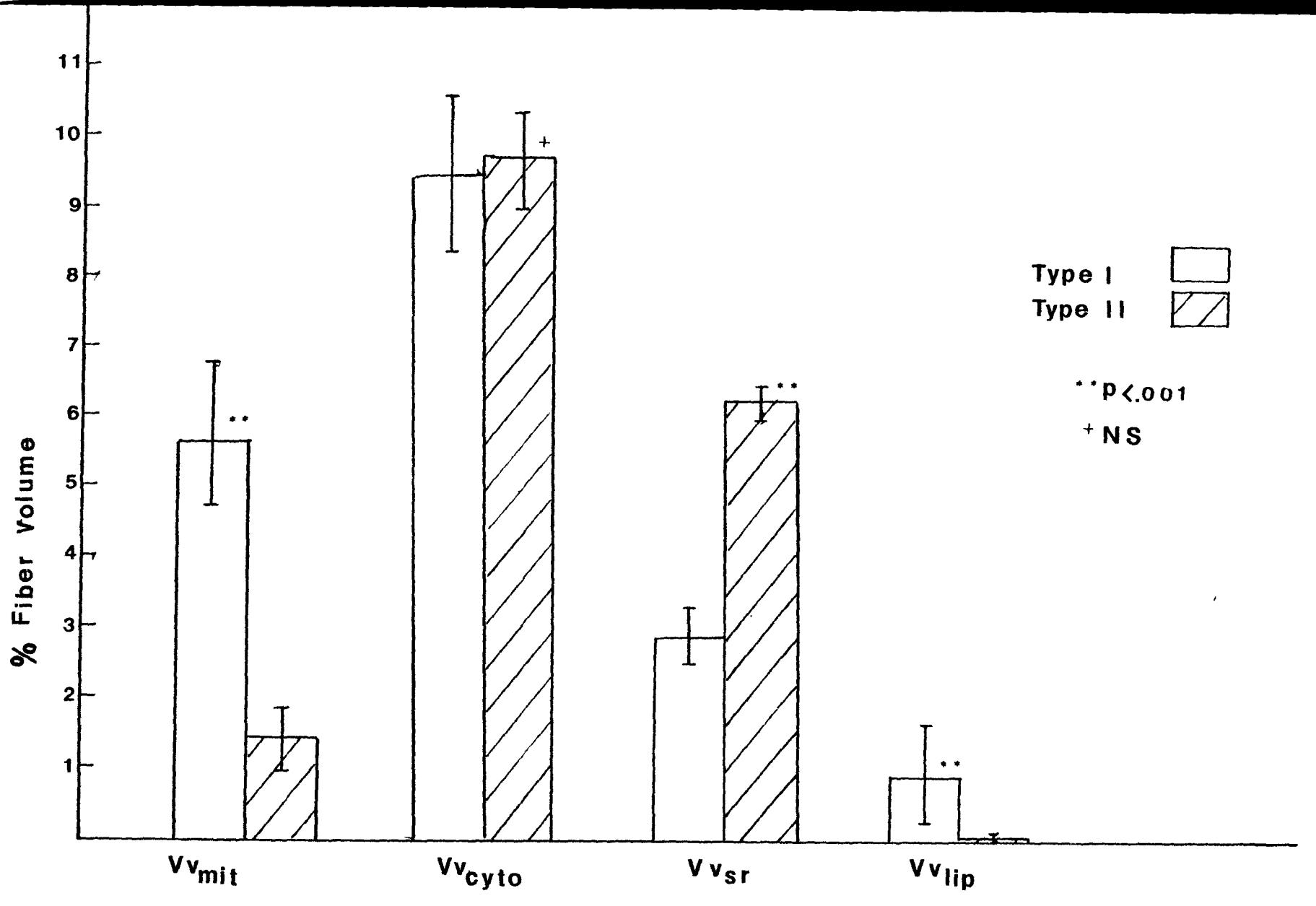


Figure 3.2 MEAN VOLUME DENSITIES FOR MITOCHONDRIA, CYTOPLASM, SARCOPLASMIC RETICULUM AND LIPID

Table 3.1

Combined Subject Mean Data $\bar{X}(\pm SD)\%$

	Type I	Type II	Sig. Level
Vv_{myof}	80.73 ($\pm$1.54)	82.48 ($\pm$1.31)	$p < .02$
Vv_{mit}	5.78 ($\pm$.978)	1.43 ($\pm$.508)	$p < .001$
Vv_{cyt}	9.48 ($\pm$1.50)	9.55 ($\pm$.752)	NS
Vv_{lip}	0.981 ($\pm$0.756)	0.037 ($\pm$0.013)	$p < .001$
Vv_{sr}	2.99 ($\pm$.86)	6.26 ($\pm$.320)	$p < .001$

Figure 3.3 MEAN MITOCHONDRIAL VOLUME DENSITY OF EACH SUBJECT

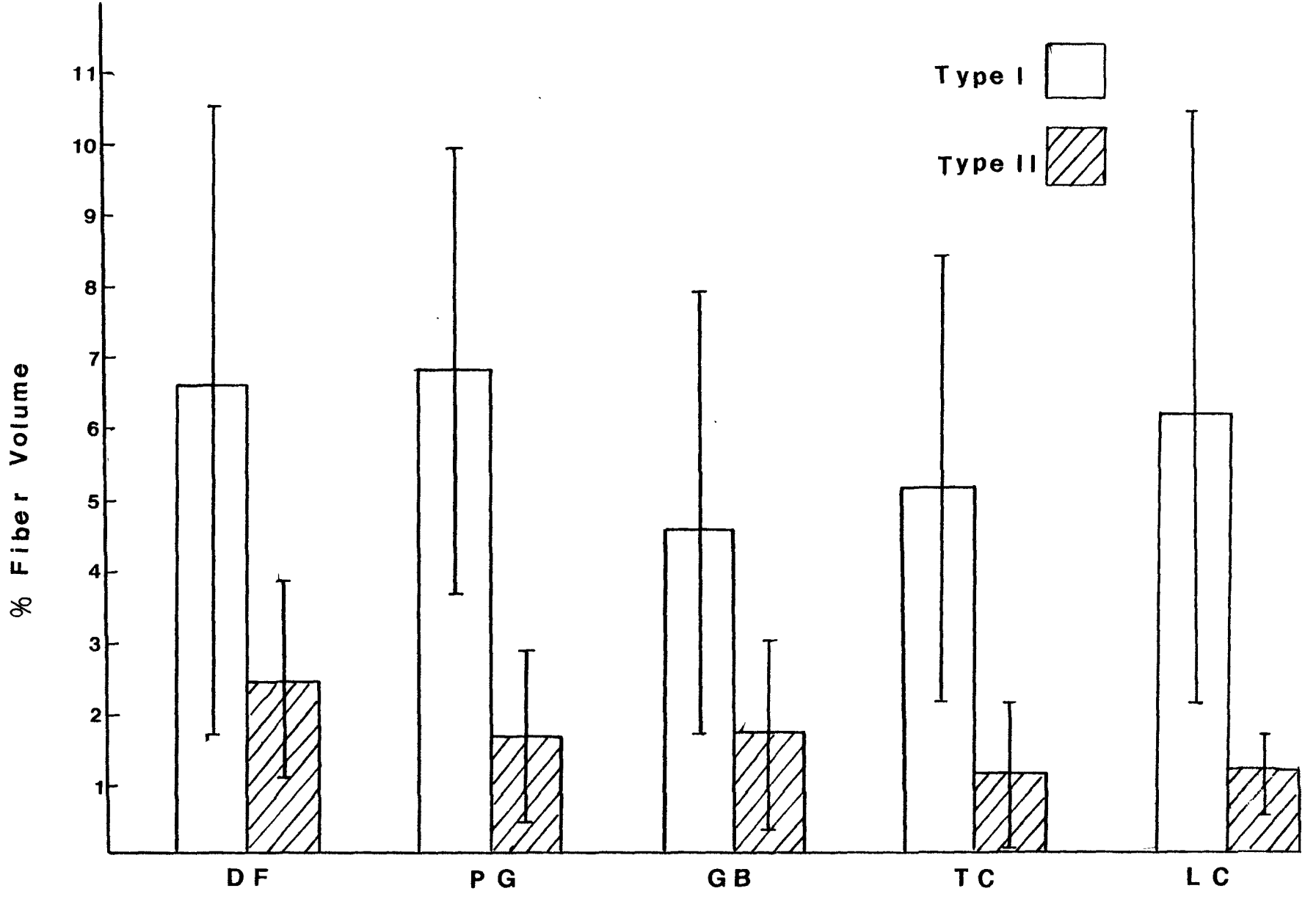
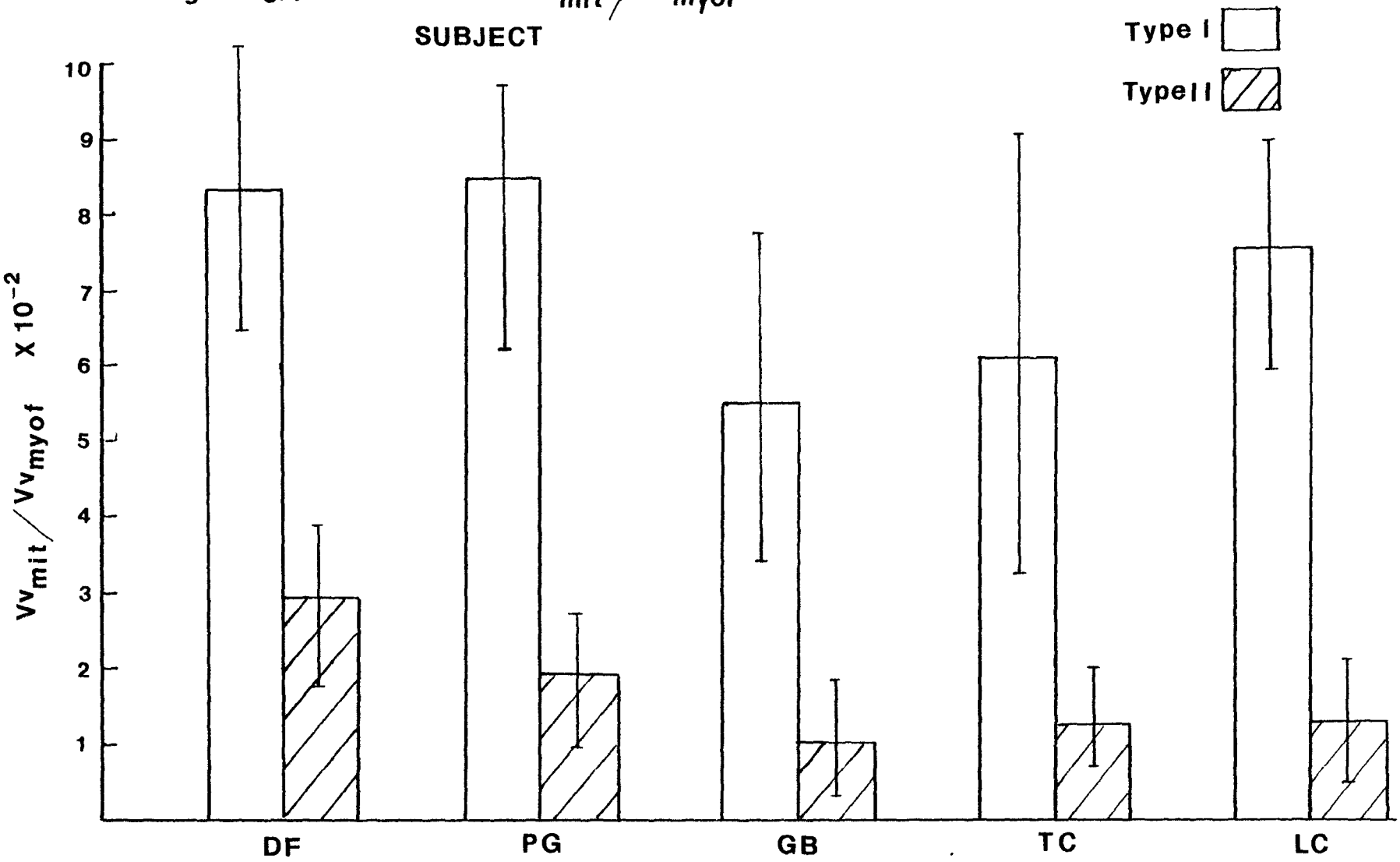


Figure 3.4 MEAN Vv_{mit} / Vv_{myof} RATIO FOR EACH SUBJECT



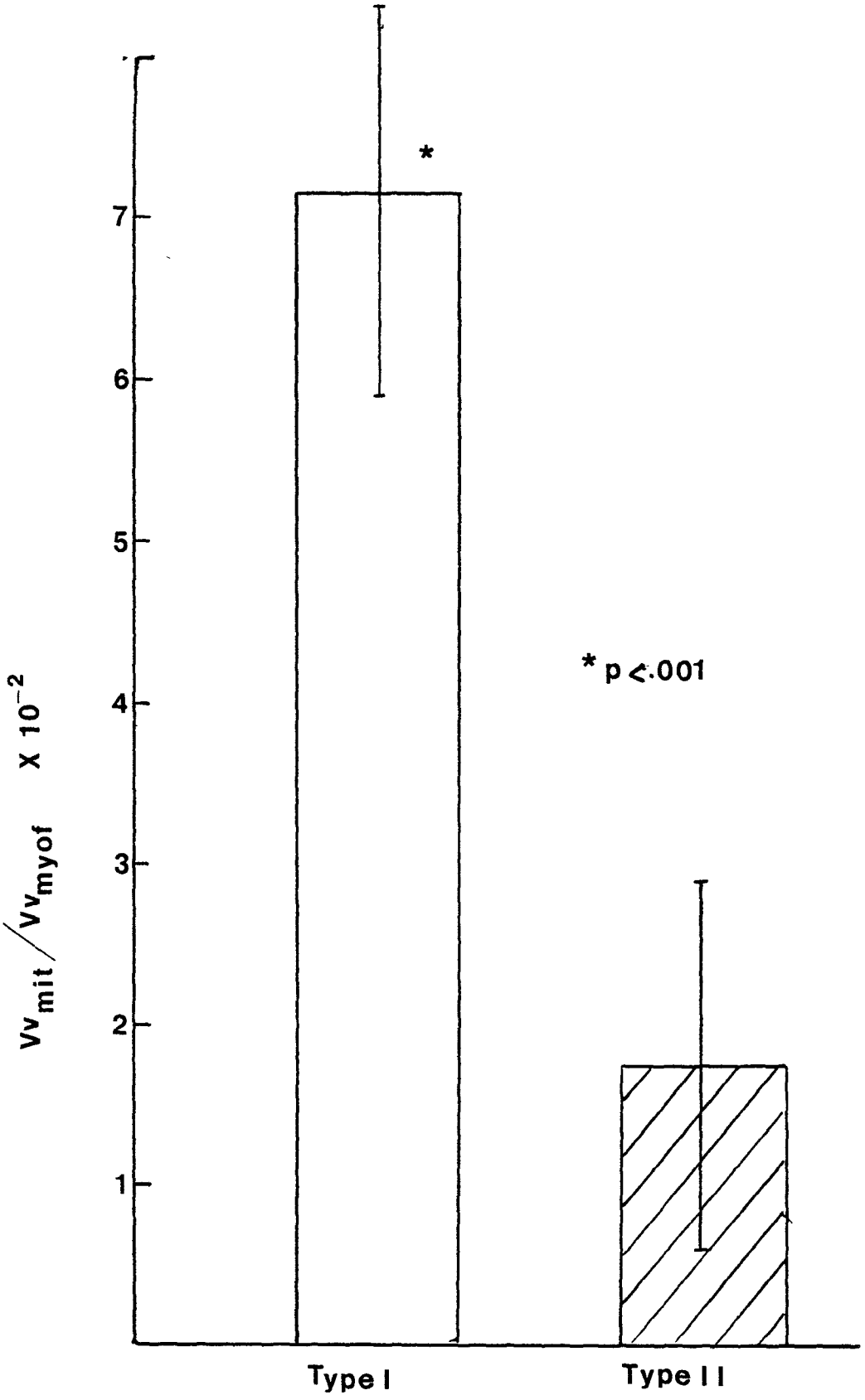


Figure 3.5 MEAN GROUP Vv_{mit} / Vv_{myof} RATIO

type II fibers (coefficient of variance 35.5). The greatest type II Vv_{mit} was found in subject DF (2.38%) and the lowest Vv_{mit} was observed in subject TC (1.08%).

The ratios of Vv_{mit}/Vv_{myof} are given in Appendix V and Figure 3.4 for each subject. The combined ratios are recorded in Table 3.2 (p. 76) and Figure 3.5 (p.80). When all subjects were considered as a single group, Vv_{mit}/Vv_{myof} was 7.18 ($\pm$1.32) x 10^{-2} and 2.71 ($\pm$1.20) x 10^{-2} in type I and II fibers respectively ($p < .001$). There were also significant inter subject differences in this ratio, for both fiber types (coefficient of variance for type I and II fibers were 18.39 and 44.2 respectively).

3.III Cytoplasmic Volume Density

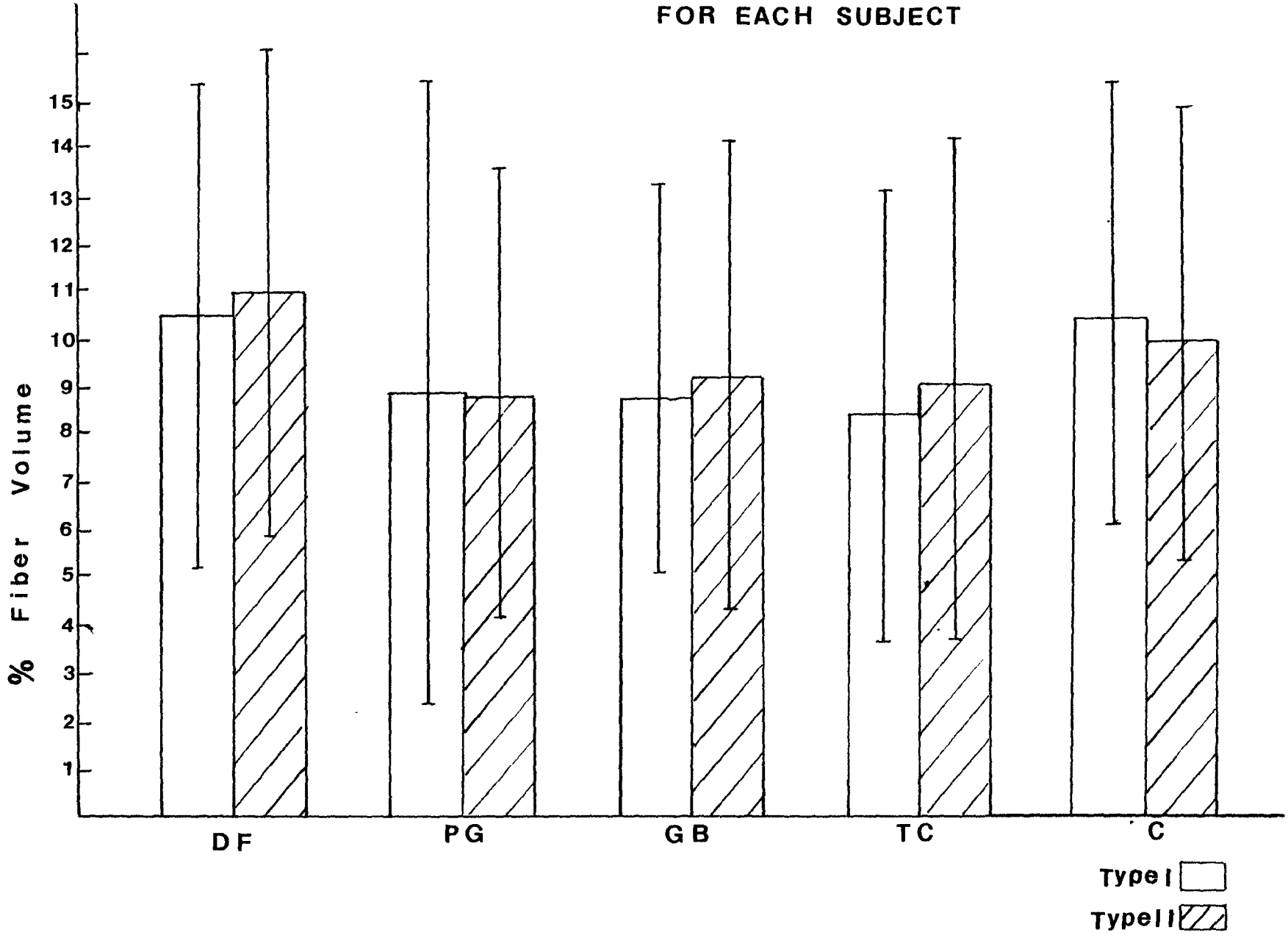
Vv_{cyt} for each fiber type are illustrated for each subject in Figure 3.6 (p. 82) and Appendix VI. Mean Vv_{cyt} of the subjects when considered as a single group is given in Figure 3.2 (p. 76) and Table 3.2 (p.84). Vv_{cyt} was not significantly different between type I (9.48 $\pm$1.50%) and type II (9.55 $\pm$.752%) fibers.

The type II Vv_{cyt} data was homogeneous among all subjects (coefficient of variance was 7.9). Type I Vv_{cyt} had significant differnnces between subject LC and TC ($p < .01$); PG and DF ($p < .02$); and GB and LC ($p < .05$). Coeefficient of variance for type I Vv_{cyt} was 15.8.

3.IV Lipid Volume Density

Vv_{lip} for each fiber type is recorded for each subject in Appendix VII and Figure 3.7 (p.83). The combined subject means are given in Table 3.1 (p.77) and Figure 3.2 (p.76).

Figure 3.6 MEAN CYTOPLASM VOLUME DENSITY FOR EACH SUBJECT



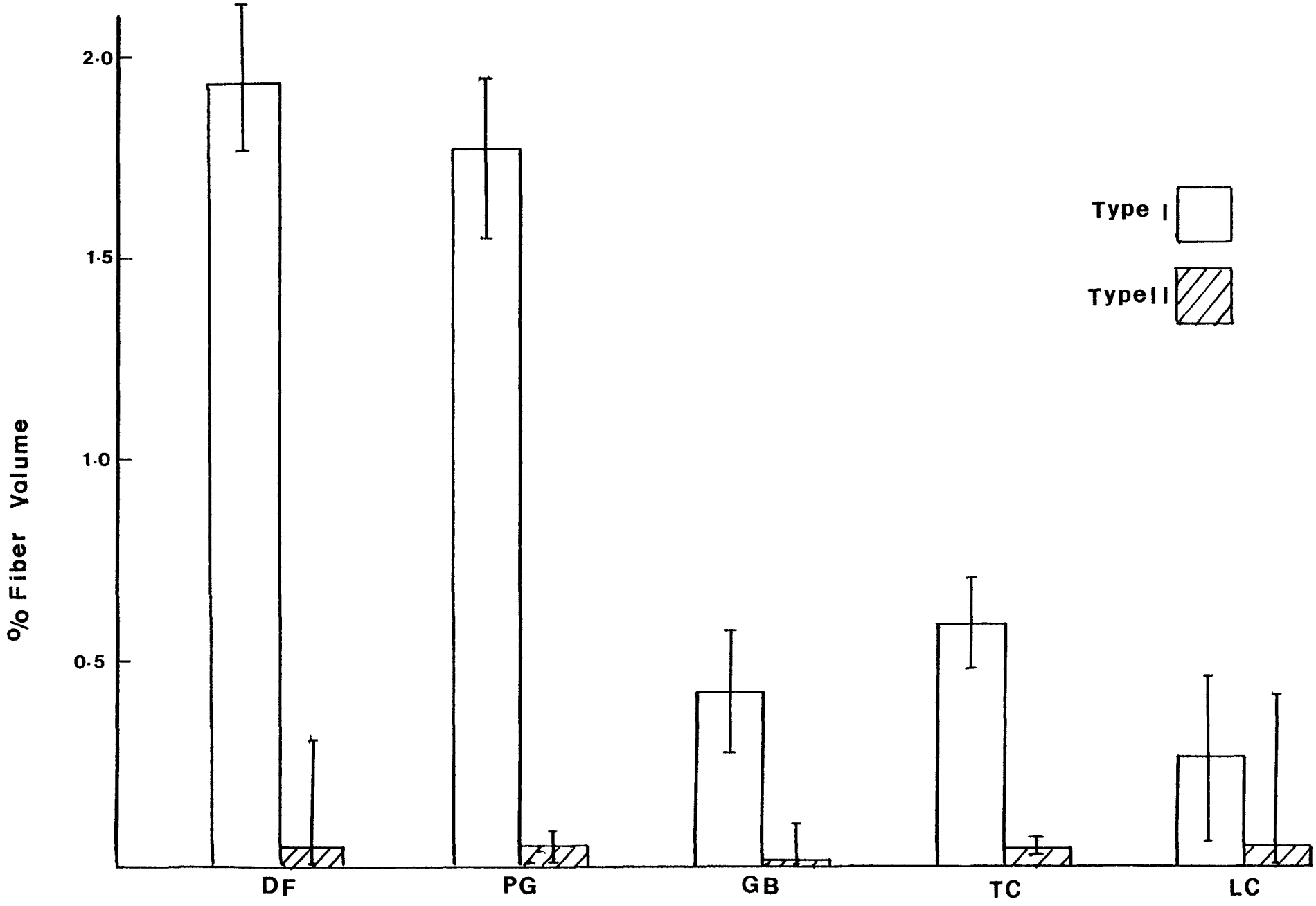


Figure 3.7 MEAN LIPID VOLUME DENSITY FOR EACH SUBJECT

Table 3.2

Combined Subject Organnelle/Myofibrillar Volume Ratio $X10^{2}$

	Type I	Type II	Sig. Level
Vv_{mit}/Vv_{myof}	7.18 ($\pm$1.32)	2.71 ($\pm$1.20)	$p < .001$
Vv_{sr}/Vv_{myof}	3.69 ($\pm$.577)	7.38 ($\pm$.269)	$p < .001$

Type I fibers had significantly greater Vv_{lip} than type II fibers at, 0.981 ($\pm$0.756)% and 0.0376 ($\pm$0.013)% respectively ($p < .001$).

Type I inter subject Vv_{lip} data showed a large coefficient of variance (77.0), however, Type II fiber Vv_{lip} showed less dispersion of data (coefficient of variance 3.45).

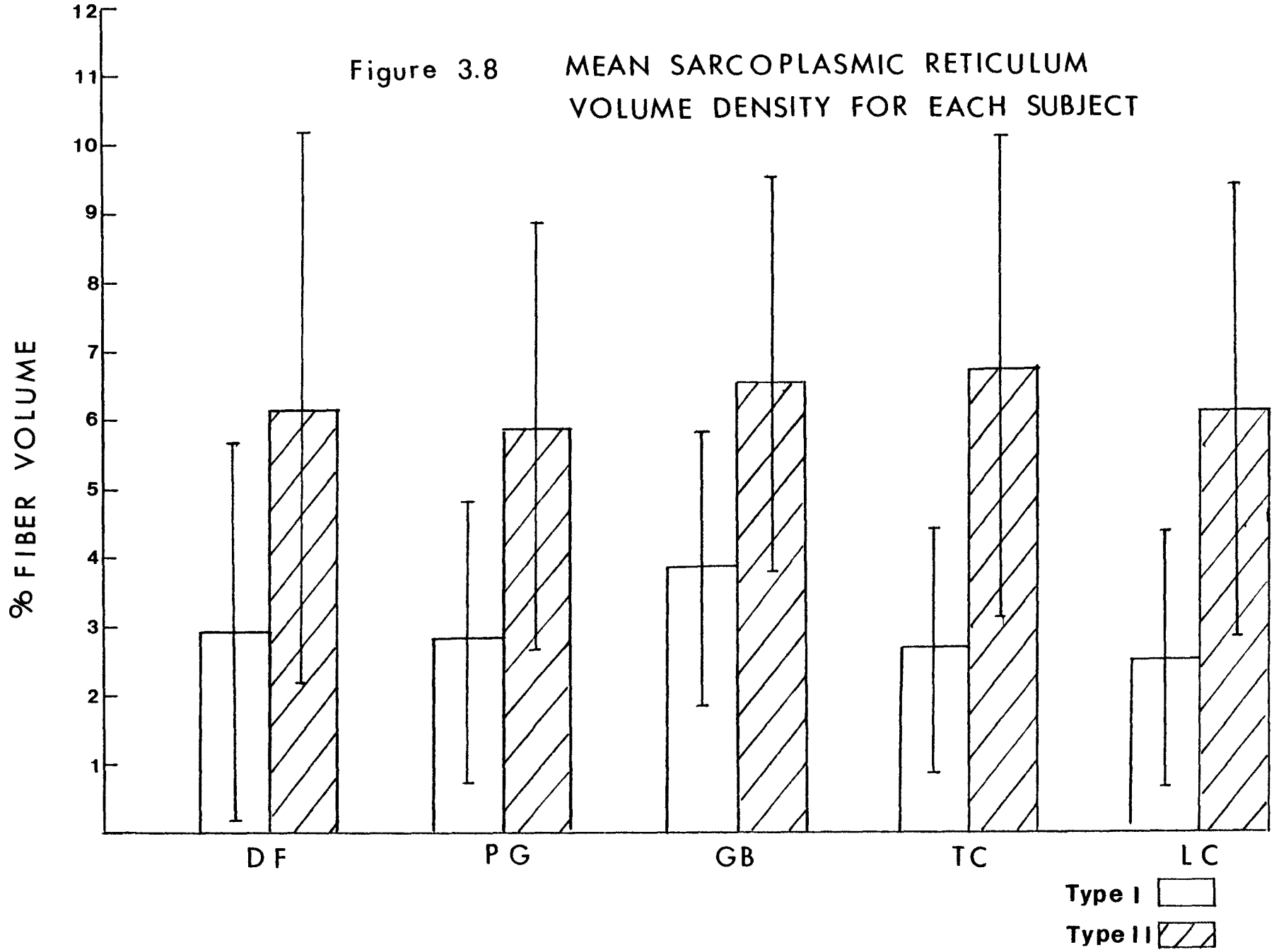
3.V Sarcoplasmic Reticulum Volume Density

Vv_{sr} for each fiber type is illustrated for each subject in Figure 3.8 (p. 86) and Appendix VIII. The combined mean data is given in Figure 3.2 (p. 76) and Table 3.1 (p. 77).

Major differences ($p < .001$) were observed in Vv_{sr} of type I and II fibers where values were found to be 2.99 ($\pm$.486)% and 6.26 ($\pm$.320) respectively. Vv_{sr} was quite consistent among type II fibers, with the only difference ($p < .02$) between subjects TC and PG. (Coefficient of variance was 5.1%). Type I Vv_{sr} was homogeneous except for subject GB whom exhibited greater Vv_{sr} values ($p < .01$) than any of the other subjects. (Coefficient of variance was 16.3).

The ratios of Vv_{sr}/Vv_{myof} for each fiber type are recorded for each subject in Figure 3.9 (p. 87) and Appendix IX. The means of the subject ratios are given in Figure 3.10 (p. 88) and Table 3.2 (p. 84). Values for Vv_{sr}/Vv_{myof} were 3.69 ($\pm$.577) x 10^{-2} and 7.38 ($\pm$.269) x 10^{-2} in type I and II fibers. This represented a significant statistical difference ($p < .001$) between type I and II fibers.

Figure 3.8 MEAN SARCOPLASMIC RETICULUM VOLUME DENSITY FOR EACH SUBJECT



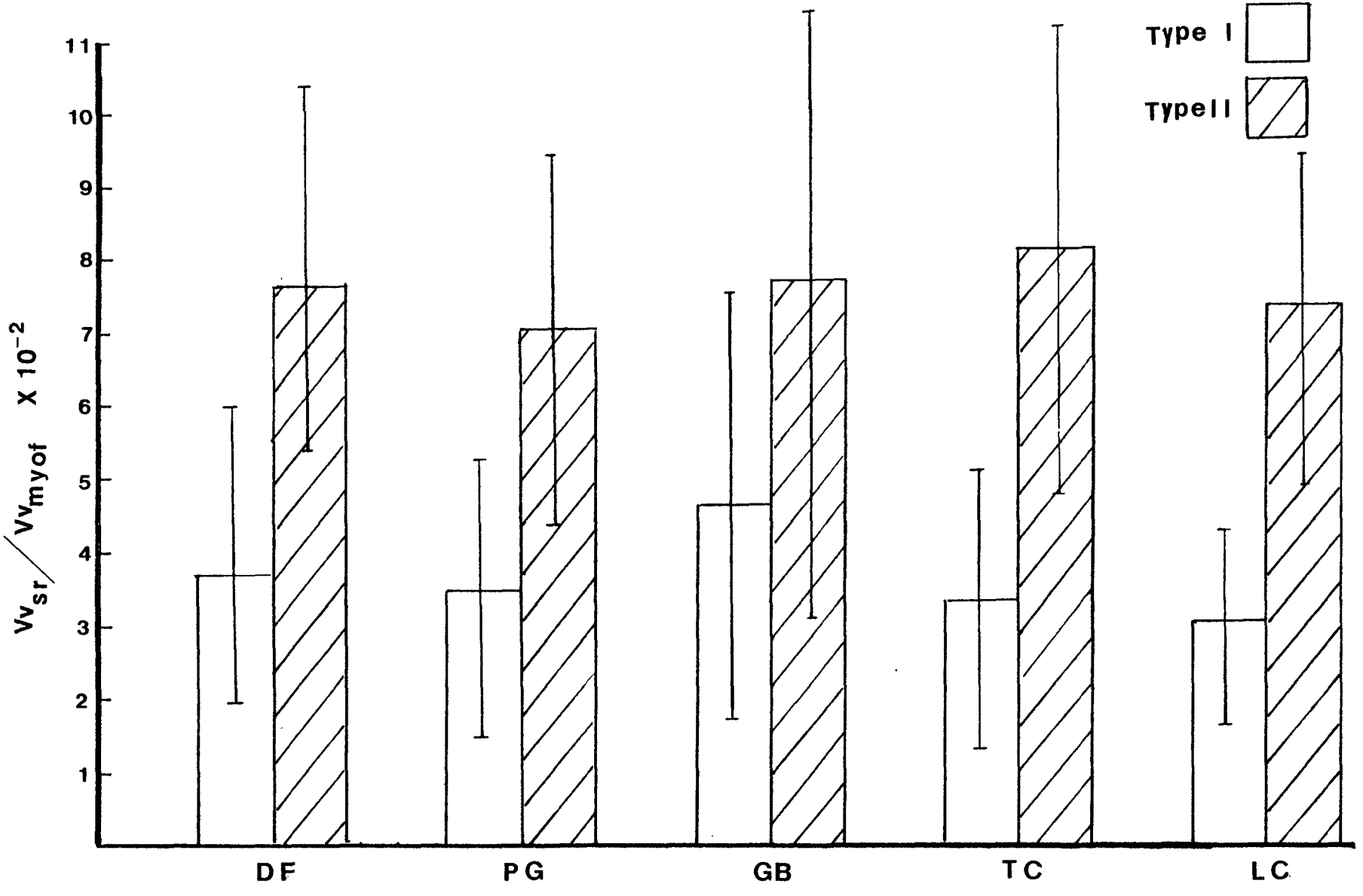


Figure 3.9 MEAN Vv_{sr} / Vv_{myof} RATIO FOR EACH SUBJECT

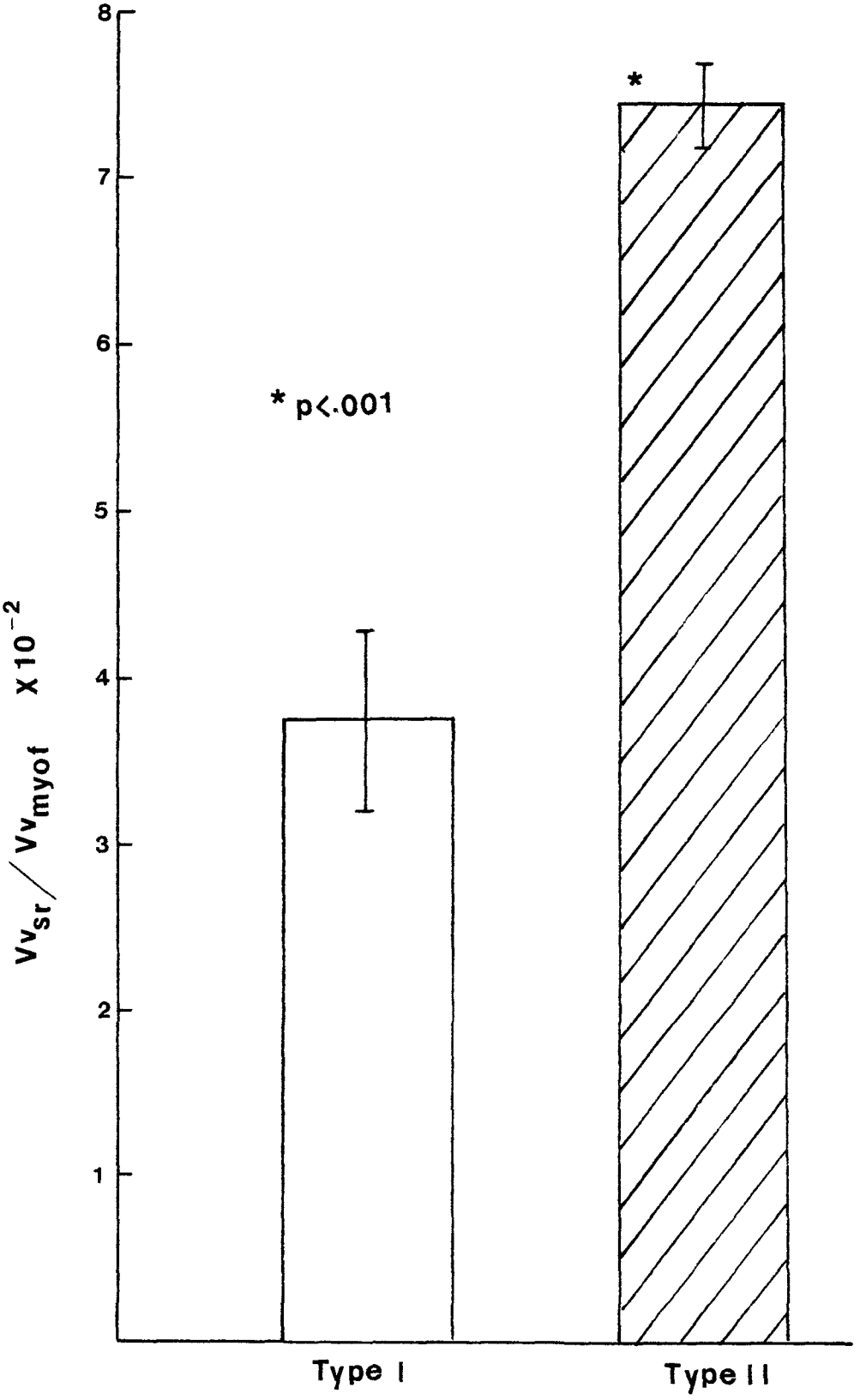


Figure 3.10 MEAN GROUP Vv_{sr} / Vv_{myof} RATIO

3.VI Fiber Areas

Fiber areas for each fiber type are recorded for each subject in Figure 3.11 (p. 90) and Appendix X. Fiber areas for subjects treated as a single group are illustrated in Figure 3.12 (p. 91) and Table 3.3 (p. 92).

When all subjects were considered as a single group, type II fibers were considerably larger than type I fibers, with mean areas of 69.84 ($\pm$17.87) x 100μm^2 and 59.87 ($\pm$16.32) x 100μm^2 respectively ($p < .01$). There was a large coefficient of variance for the grouped data of both fiber types (27.3 and 25.6% of type I and II fibers respectively). Only subject PG and TC had similar type I fiber areas.

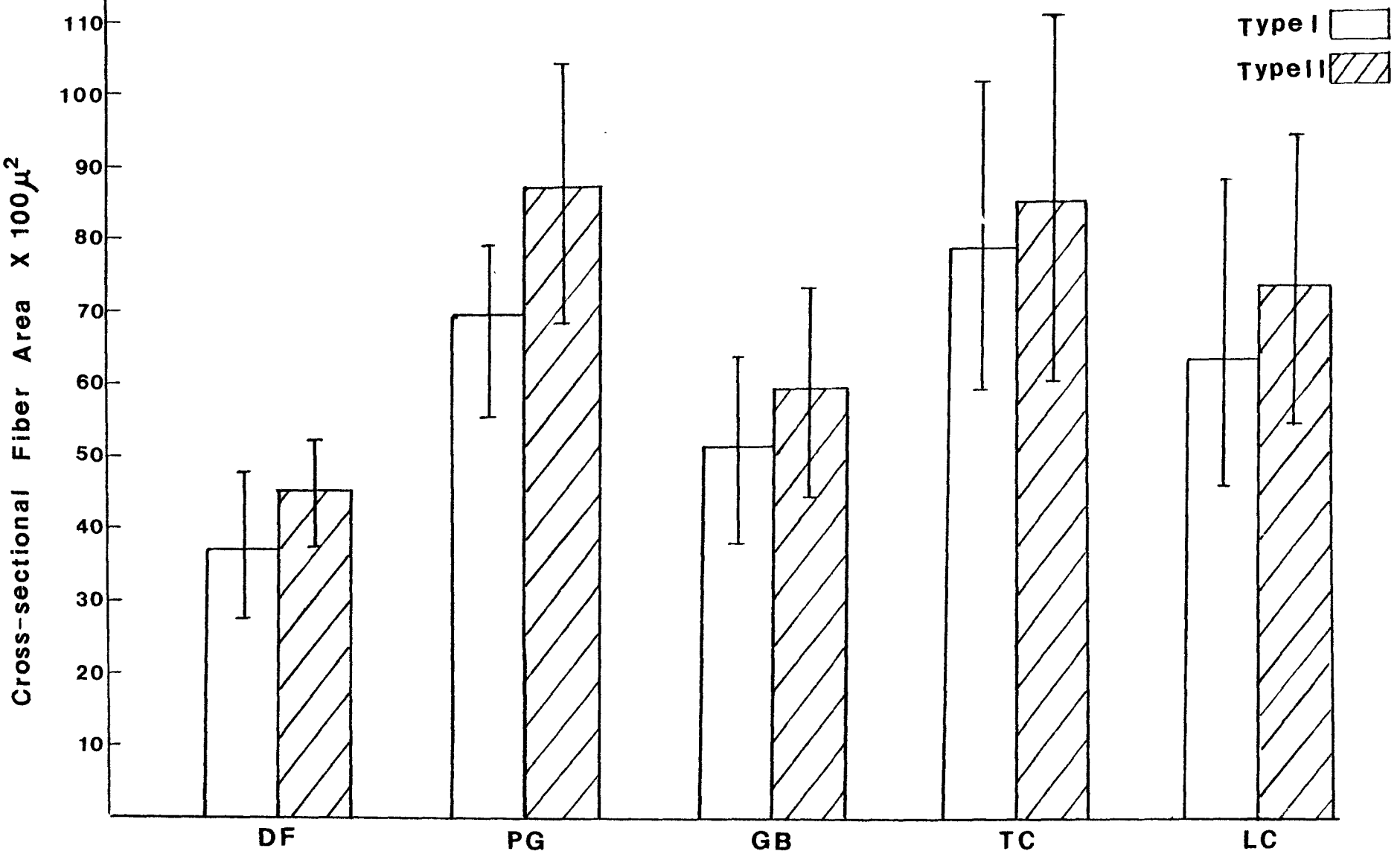
3.VII Myofilament Packing Density

Myofilament packing density for each subject is illustrated in Figure 3.13 (p.93) and Appendix XI. Combined subject data for both fiber types is found in Figure 3.14 (p. 94) and Table 3.4 (p. 92).

The subjects when treated as a single group showed no significant difference ($p < .10$) between myofilament packing densities. The data is recorded as the number of myosin filaments per .5μm^2. Myosin packing density was 140.38 ($\pm$3.82)/.5μm^2 in type I fibers and 140.76 ($\pm$3.93)/.5μm^2 in type II fibers.

Individual subject data is recorded for 30 fibers of each type in Appendix XI. There was a small coefficient of variance for the grouped data of both fiber types (2.72 and 2.79%, respectively).

Figure 3.11 MEAN FIBER AREA FOR EACH SUBJECT



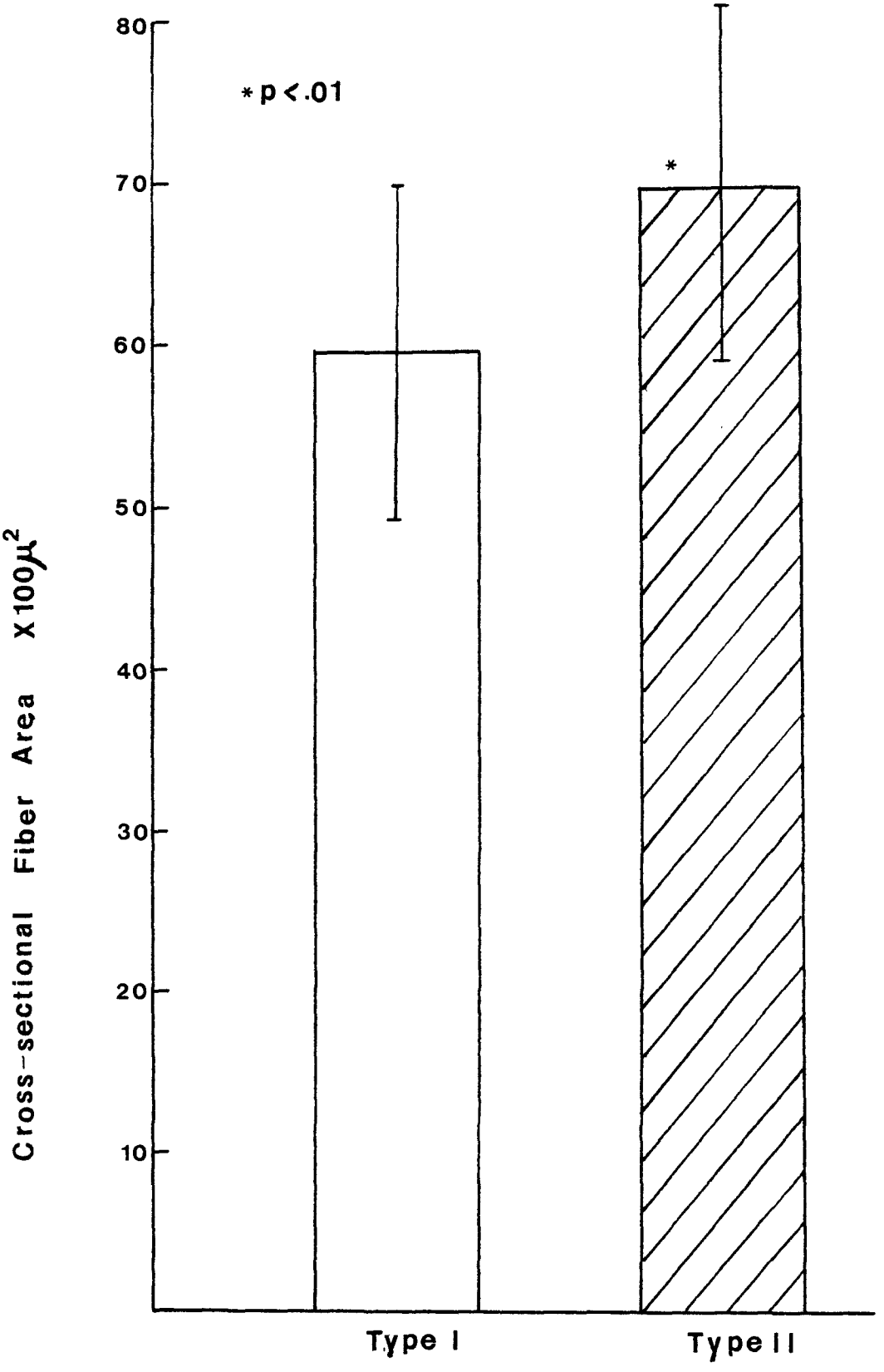


Figure 3.12 MEAN GROUP FIBER AREA

Table 3.3

Combined Subject Area Measures ($\bar{X} \pm SD$) x $10^2 \mu^2$

	Type I	Type II	Sig. Level
Fiber Areas	59.87 ($\pm$16.32)	69.84 ($\pm$17.87)	$p < .01$

Table 3.4

Combined Subject Myofilament Packing Density $\bar{X}(\pm SD)/.5\ \mu^2$

	Type I	Type II	Sig. Level
Packing Density	140.38 ($\pm$3.82)	140.76 ($\pm$3.93)	NS

Figure 3.13 MEAN MYOFILAMENT PACKING DENSITY FOR EACH SUBJECT

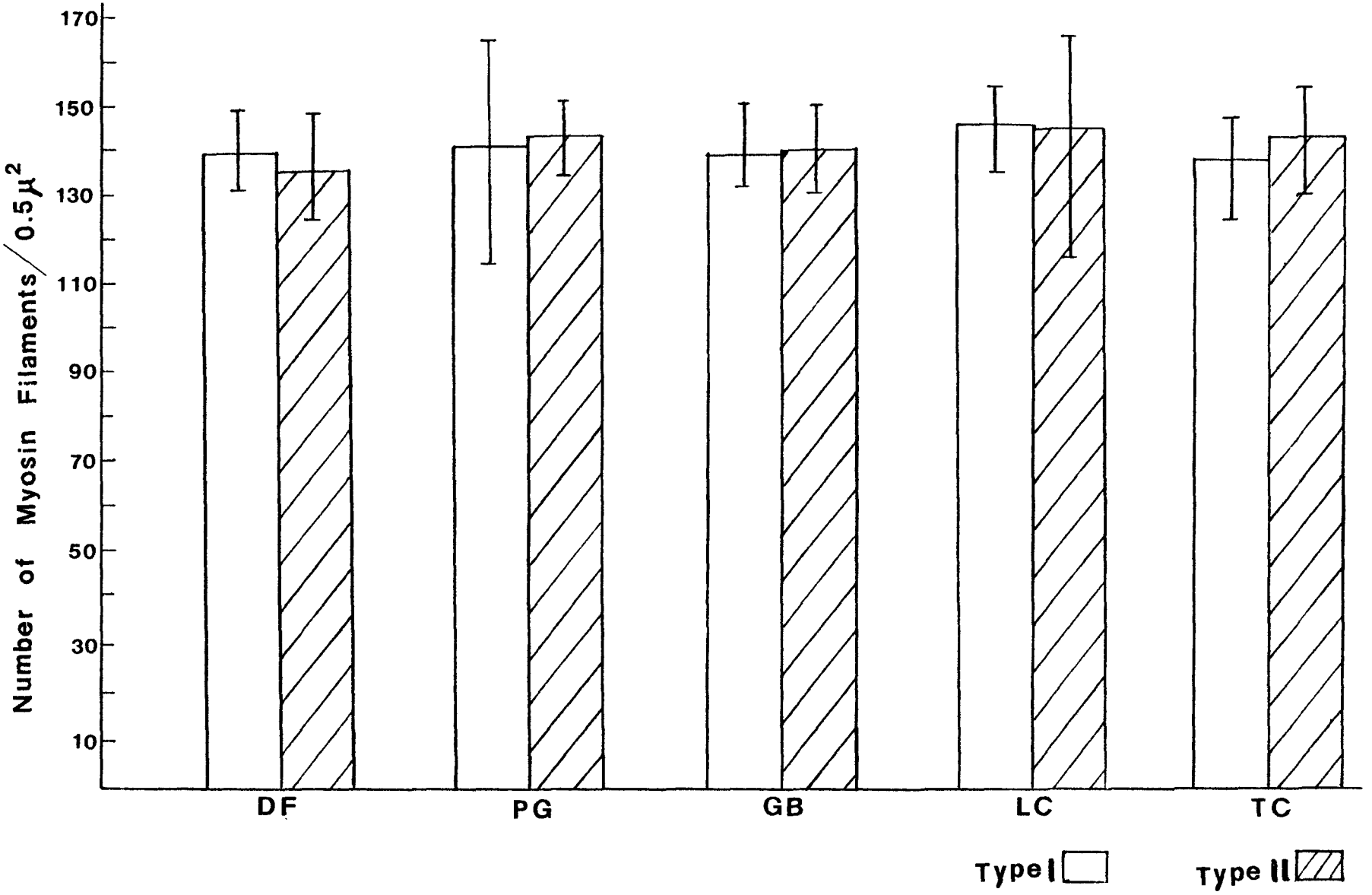
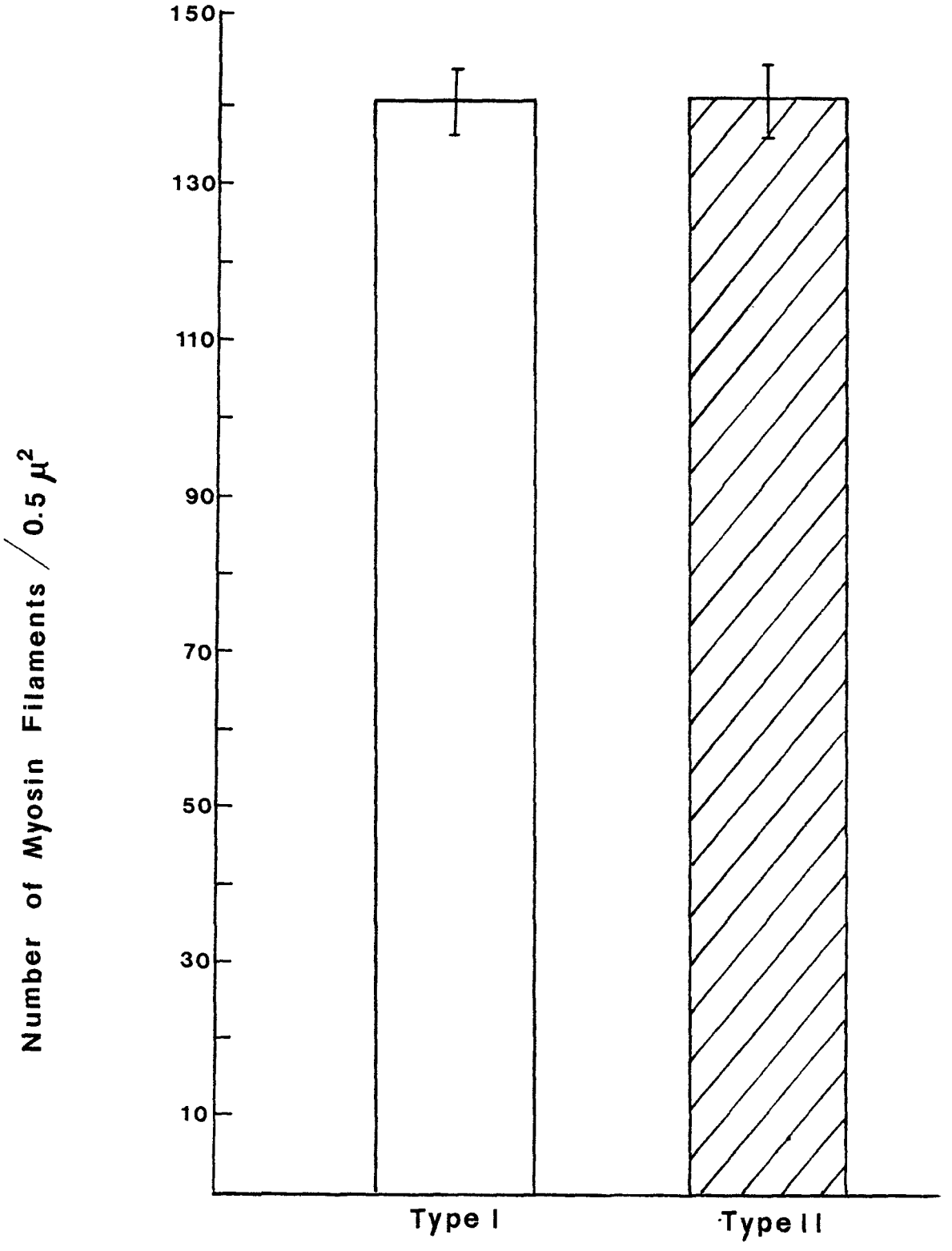


Figure 3.14 MEAN GROUP MYOSIN PACKING DENSITY



CHAPTER 4

A. Discussion

4.I Introduction

Ultrastructural characteristics of type I and II fibers were determined from 50 fibers of each type from each of five subjects. Type I and II fibers from human vastus lateralis were found to possess both similar and distinctly different characteristics. This chapter will discuss the morphometric characteristics according to fiber type.

4.IIA Myofibrillar Volume Density

When all subjects were considered as a single group, myofibrillar volume density was found to be significantly ($p < .02$) greater in type II than type I fibers (82.48% and 80.73% respectively).

The values obtained are similar to previous human Vv_{myof} studies from the vastus lateralis (Table 4.1; p. 97).

Despite the fact that animal studies have compared two different muscles with different functions, angles of insertion and anatomical location, the Vv_{myof} of such investigations were found to be similar to the Vv_{myof} of human vastus lateralis.

4.IIB Differences in Myofibrillar Volume Density

It is known that type II fibers can produce a greater tension per unit of cross sectional area, than type I fibers (Burke and Tsairis, 1973; Burke and Edgerton, 1975). This study has shown that type II fibers have a greater Vv_{myof}, and therefore a greater percentage of a given cross sectional area will be represented as contractile protein. It is also

recognized that the myofibrillar protein is responsible for the production of tension (by the actin and myosin interactions). Therefore, it is not surprising that a fiber with a greater Vv_{myof} and thus a greater percentage of contractile protein per unit area (i.e. type II fibers) would produce a greater force than a fiber containing less contractile protein per unit area (i.e. type I fibers). In conclusion, the greater force production which is observed in type II fibers may be accounted for by a greater Vv_{myof}.

4.IIIA Mitochondrial Volume Density

When the subjects were considered as a single group Vv_{mit} was found to be significantly greater ($p < .001$) in type I than type II fibers (5.78% and 1.43% respectively).

Vv_{mit} is known to vary between different muscles, and probably reflects both the level of training and the fiber type distribution of the muscle. Previous animal work has reported significant differences between fiber types (Stonnington and Engel, 1973; Tomanek et al, 1973; Eisenberg and Kuda, 1976). However, the human studies were unable to classify different fiber types, and therefore reported the mean values of both type I and II fibers. Their results would therefore be influenced by the number of each fiber type which was studied. The studies which have used longitudinal samples, have found greater Vv_{mit} levels in type I fibers, but have found a considerable overlap between the fiber types (Ogata and Murata, 1969; Cullen and Weightman, 1975; Payne et al, 1975). This overlap may be the result of the inaccurate method of M or Z line measurement which was used for fiber typing by these authors (Jerusalem, 1974; Cullen and Weightman, 1975).

Table 4.1

Summary of Previous Ultrastructural Results

	% Fiber Volume					
	Vv_{myof}	Vv_{mit}	Vv_{cyt}	Vv_{lip}	Vv_{sr}	Packing Density /0.5μ^2
a. Vastus Lateralis						
i) Penman (1969)	-	-	-	-	-	128
ii) Hoppeler et al (1973)	85.87	5.19	8.90	0.34	3.78	-
iii) Cullen and Weightman (1975) (longitudinal)	-	5.14	-	0.63	9.37	-
iv) Howald (1976)	77.94	-	-	-	-	-
v) Bell et al (1980) (boys)	82.31	5.54	11.50	0.55	-	-
b. Triceps						
MacDougall et al (1979)	82.25	3.04	14.54	0.12	-	-

4.IIIB Differences in Mitochondrial Volume Density

The differences in Vv_{mit} found in this present study were not surprising. Histochemical staining has shown greater oxidative mitochondrial enzyme activity in type I fibers, while type II fibers showed greater glycolytic enzyme activity (Brooke and Kaiser, 1970; Engel, 1974; Askanas and Engel, 1975). The oxidative enzymes of the mitochondria are responsible for providing the major pathway for ATP production in type I fibers. Type II fibers favor glycolytic pathways for energy production and therefore do not depend to the same extent upon the presence of oxidative mitochondrial enzymes (McGilvery, 1975). The greater Vv_{mit} in type I fibers than in type II fibers reinforces their reliance upon oxidative pathways for energy production.

4.IIIC Intersubject Differences

Examination of individual subject data showed a large coefficient of variation in both type I and II fibers (64.36 and 83.8% respectively). It is not surprising that there are large intersubject differences since Vv_{mit} is affected by level of aerobic conditioning (Hoppeler et al, 1973), inherited genetics (Howald, 1976) and age (Bell et al, 1980). Even monozygotic twins were found to have a mean intra pair Vv_{mit} difference of $0.46 \pm 0.37\%$ (Howald, 1976).

In summary, there are intersubject differences between Vv_{mit} levels within each fiber type, but when type I and II fibers are compared, type I fibers consistently have a significantly greater Vv_{mit} level than type II fibers.

4.IIID Mitochondrial Volume Density/Myofibrillar Volume Density Ratio

The grouped Vv_{mit}/Vv_{myof} ratio was significantly greater ($p < .001$) in type I than II fibers (7.18×10^{-2} and 2.71×10^{-2} respectively). Vv_{mit}/Vv_{myof} ratios may be a better index of the potential for the muscle to perform aerobic activity than VO_2 (MacDougall et al, 1979). Vv_{mit} and Vv_{mit}/Vv_{myof} have been shown to relate to the oxidative enzyme activities and maximal oxygen uptake (Kiessling et al, 1974; Howald, 1976). Since Vv_{mit}/Vv_{myof} accounts for the myofibrillar protein which uses the ATP from aerobic metabolism (i.e. from mitochondrial enzymes), this ratio is a valid method for expressing a muscle's potential for aerobic activity.

The mechanisms behind a greater susceptibility of type II fibers to fatigue are not clear, but may be related to Vv_{mit}. As already indicated in this present study, type II fibers had less Vv_{mit} and greater Vv_{myof} than type I fibers. Therefore, the type II fiber Vv_{mit}/Vv_{myof} ratio indicated a further "diluting" of mitochondrial content. In addition, type II fibers have a faster turnover of calcium and ATP in connection with muscular contraction as well as for ATP production via anaerobic processes. It is evident that type II fibers have a decreased fiber oxidative enzyme potential and thus a decreased potential to perform aerobic activity. Type I fibers on the other hand, have a higher aerobic potential as evidenced by a high content of mitochondria (Vv_{mit}) as well as higher activity of the mitochondrial enzymes. Failure in the excitation-contraction coupling as well as in the ATP supplying mechanisms may account for the greater susceptibility to fatigue in type II fibers.

4.IV Cytoplasm Volume Density

When all subjects were considered as a single group, there was no significant difference ($p > .05$) in Vv_{cyt} between type I and II fibers (9.48%

and 9.55% respectively). The subjects had a very small coefficient of variance for type I and II fibers (15.8% and 7.87% respectively) which indicated that Vv_{cyt} was a constant paramater among all the subjects.

Vv_{cyt} in this present study differed from the results from other authors, in that it was greater than the value reported by Hoppeler et al (1973) but less than that reported by Bell et al (1980). A possible explanation for a slightly higher Vv_{cyt} in this study, might be a different fixative concentration and an additional freezing process, which may have caused expansion of cytoplasm leading to slightly exaggerated levels. However, if this did occur it must be assumed that it would be a constant error in both fiber types, thereby still ensuring the validity of comparison of type I and II fibers.

The study by Bell et al (1980) used identical tissue processing to Hoppeler et al (1973) but yielded a much greater cytoplasm volume density. However, these authors did not identify the fiber SR content and counted both SR and cytoplasm in the total Vv_{cyt} value. If 2.99%-6.26% of their Vv_{cyt} is subtracted (which are the values obtained in this study for Vv_{sr}) the cytoplasm volume density then becomes very close to the values found in this study.

Although type II fibers are often reported as being largest in size (but with considerable variation depending upon the muscle), no evidence was found in this study to suggest that this greater size was due to an increased cytoplasmic volume. There were considerable differences in fiber sizes between subjects yet Vv_{cyt} were similar.

4.VA Lipid Volume Density

The group Vv_{lip} was found to be significantly greater in type I than type II fibers (0.981% and 0.037% respectively).

Table 4.2

Summary of Previous Animal Studies

	Rat		Guinea Pig	
	Stonnington & Engel (1973)		Eisenberg *et al* (1974)	Eisenberg & Kuda (1975)
	I	II	I	II
Vv_{myof}	82	85	-	-
Vv_{mit}	-	-	4.86	1.91
Vv_{lip}	-	-	.20	.012
Vv_{sr}	-	-	2.99	4.59

The results of this study agree with previous animal and human research (see Table 4.2), although the absolute volume densities were found to differ between different animals and muscles.

4.VB Differences in Lipid Volume Density

The deposition of lipid droplets are often found in close proximity to mitochondria (Fawcett, 1966). Lipid oxidation via the citric acid cycle is the most fuel-efficient pathway for ATP production (Morgan et al, 1969; McGilvery, 1975). Consequently, one would expect more lipid in type I fibers since there is a greater Vv_{mit} and enzyme activity for oxidation of fatty acids. Type II fibers generally require a faster turnover of ATP for contraction, and therefore rely very little on fatty acid oxidation, which is a slower process (McGilvery, 1975). In addition one could expect that the low Vv_{mit} in type II fibers prevents extensive lipid oxidization, due to the lower level of mitochondrial lipid-oxidization enzymes.

4.VIA Sarcoplasmic Reticulum Volume Density

When subjects were considered as a single group, type II fibers were found to have a significantly greater ($p < .001$) Vv_{sr} than type I fibers (6.26% and 2.99% respectively).

Previous animal studies have shown similar results (Table 4.2) to those presented in this study. Studies from human muscle were unable to distinguish between fiber types due to methodological problems. As a results, these authors reported values which were approximately equal to the mean of the type I and II Vv_{sr} reported in this present study (see Table 4.1).

4.VIB Sarcoplasmic Reticulum Volume Density to Myofibrillar Volume Density Ratio

When all subjects were pooled as a single group, type II fiber Vv_{sr}/Vv_{myof} ratio was found to be significantly greater ($p < .001$) than the type I ratio (7.38×10^{-2} and 3.69×10^{-2} respectively).

The ratio of Vv_{sr}/Vv_{myof} is important because it relates the potential release of calcium ions per unit of myofibrillar contractile protein. A larger Vv_{sr}/Vv_{myof} ratio would suggest a greater potential for release and uptake of calcium. The contraction of skeletal muscle and the role of calcium in the interaction of actin and myosin will be reviewed in order to understand the significance of the SR complex.

4.VIC Interaction of Actin and Myosin and Calcium for Contraction

Myofibrillar fine structure

The basic contractile components of the muscle fiber are assembled by four proteins. Actin and myosin have previously been mentioned. The other two proteins are tropomyosin and troponin. Neither protein by itself is contractile. Tropomyosin and troponin serve a regulatory function in making and breaking the contacts between actin and myosin (Endo, 1966). Tropomyosins are long polypeptide molecules running along the length of the helix actin filament. Troponin has a globular shape and sits on the tropomyosin molecule close to one of its ends. One troponin can regulate the activity of seven actin monomers in the presence of one tropomyosin molecule (Weber, 1976).

Excitation

The motor nerve stimulates the muscle and the propagated action potential depolarizes the muscle cell membrane. There is an inward spread of the action potential along the T system (i.e. tubules are depolarized)

(Ebashi, 1976). This causes the membrane of the terminal cisternae of the SR to increase its permeability to calcium (Ebashi, 1976). At rest the sarcoplasm surrounding the myofibrils is almost free from calcium (concentration is less than 10^{-7} M). Calcium ions are released from the stores on the SR and two calcium ions become bound to each troponin molecule (Weber, 1963; Kendrick-Jones et al, 1970; Ebashi, 1976).

Contraction

The troponin bound calcium causes a change in the troponin-tropomyosin-actin complex which will remove the inhibition for an interaction between the myosin head and actin. The tropomyosin rods are moved from their blocking position. With the binding of calcium ions to troponin, the tropomyosin strands are drawn toward the center of the actin filament thus allowing actin to react with myosin; the actin monomers are now released from the pre-existing inhibitory influence of the troponin-tropomyosin complex (Huxley, 1972). According to Huxley's (1972) theory, the heads of myosin molecules move out perpendicular from the thick filament core toward the actin filament and attach (the S-1 units) to the actin molecules within reach. The cross-bridges with attached M-ATPase are then bound to actin. The cross bridges undergo an energy-yielding positional change so that the cross bridges change their angular relationship to the axis of the myosin core. The actin filaments are pulled along the myosin. These events are repeated as long as the muscle is stimulated and the cross-bridges attach, swivel and detach cyclically, thus propelling the thin filaments past the thick ones shortening the muscle (Huxley, 1972).

Relaxation

Fresh ATP is taken up by the myosin head which will dissociate actin from myosin. Calcium is released from the troponin and taken up by

the membranes of the SR (Ebashi, 1976). The tropomyosin changes to its original position relative to the actin subunits and again inhibits actin from interaction with the cross bridges. Finally, the cross bridges move back to their original position.

4.VID Difference in Sarcoplasmic Reticulum Volume Densities

The physiological significance of a greater Vv_{sr} in type II fibers is probably related to the calcium release and uptake functions of the SR. It is known that calcium must be released by the SR in order to allow actin and myosin interaction, but the concentration of calcium needed to initiate a contraction has not been determined (Podolsky, 1969; Podolsky and Teicholz, 1970; Endo, 1972).

Surface density for SR membrane was not measured in this present study, however presumably it was greater in type II fibers than in type I fibers. One might hypothesize that a fiber which possesses a greater SR membrane surface area would be capable of binding a greater total quantity of free calcium ions than a fiber with a lower SR membrane surface area. Thus a greater potential release and uptake of free calcium would be possible in those fibers with a high SR content.

A greater calcium release and thus greater calcium concentrations may in part account for increased tensions per area which have been observed in type II fibers (Burke and Tsairs, 1974; Burke and Edgerton, 1975). Support for this hypothesis has been observed in several animal studies which have shown that varied concentrations of calcium within a muscle or fiber influence the production of tension. Skinned frog fibers were found to increase tension steeply over 10 to 90% of peak force with a small increase of calcium (Hellman and Podolsky, 1969; Podolsky and Noland,

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Appendix I

Diaminobenzidine-Peroxide Solution

1. 15.0 mg of 3.3' diaminobenzidine tetrachloride (DAB) were placed in 4.0 ml of distilled H_2O then agitated over a moderate heat until dissolved.
2. 157.6 mg of trio HCl buffer and 6 ml of distilled H_2O were added to the DAB mixture. Agitation of the solution was continued but the heat was removed.
3. 10.0 ml of 0.2% fresh hydrogen peroxide was added to the above solution.
4. The final 20 ml solution was pHed to 7.0 then filtered with number 1 Whatman filter paper.
5. The filtered solution was placed in the coplin jars which contained slides of fixed tissue.
6. The coplin jars were placed in a water bath at 37°C for 30 minutes to allow the reaction to occur.
7. The stained sections were removed then dehydrated and embedded for electron microscopy.

Appendix II

Validation of Technique

To establish the validity of the myoglobin staining technique for identifying skeletal muscle fiber types, the same fibers were stained for both myoglobin and ATPase activity (Brooke and Kaiser, 1970). Needle biopsies from the vastus lateralis of three subjects who had given written consent were frozen, then serially sectioned in a cryostat. These sections were stained for M-ATPase activity following preincubation pH's of 10.0, 4.6 and 4.3 (modified Brooke and Kaiser, 1970), and an oxidative enzyme stain of NADH-TR (Novkoff et al, 1961). The remaining tissue sample was immersed in 2% glutaraldehyde for four hours at 0°C, to fix the fine structure. Following a careful washing of the sample in 2 M sodium cacodylate buffer to remove all glutaraldehyde, the sample was refrozen and cut on a cryostat. It was our experience that freezing tissue after fixation caused minimal artifact. However, freezing the sample prior to fixation (as was necessary in this validation study, to compare myoglobin and histochemical reactions on the same fibers) caused considerable damage. The fixed sections were stained for myoglobin by a diaminobenzidine-peroxidase reaction (See Appendix I).

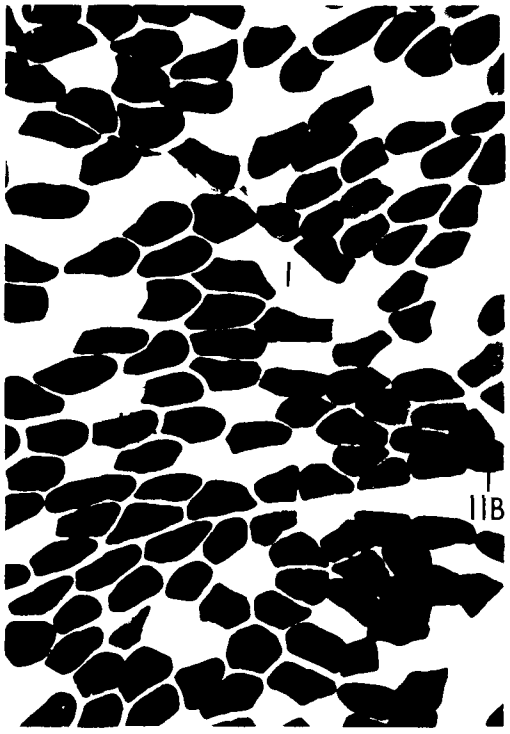
The same field of fibers was identified in the M-ATPase, NADH-TR and myoglobin reactions by a light microscope, and light micrographs were taken of the same fields in all reactions. Fibers were classified according to easier individual histochemical reactions. For example, fibers from all the light micrographs from the ATPase reactions with 10.0 pH preincubations were classified as either type I or type II (in some cases, type II subunits could be identified). The other two M-ATPase reactions were examined in a

similar manner until all of the fibers were designated as either type I, IIA or IIB fibers. Since type IIC fibers are rare in untrained persons, the numbers would have been too low to establish any conclusion concerning this fiber type and the myoglobin reaction. As a result any type IIC fiber and, any fiber which was unclassifiable by the M-ATPase technique was not included in the final cross-comparison with the myoglobin stain. This amounted to nine fibers out of the 951 which were examined. Fiber identification was made directly on the light micrographs.

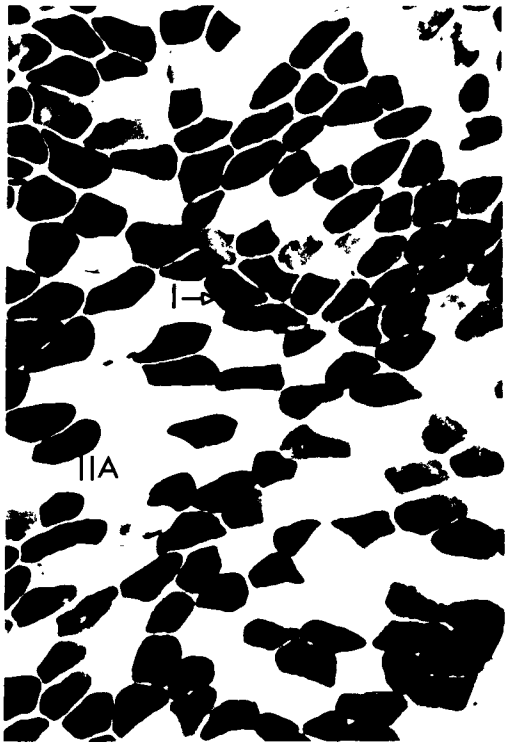
Finally, the fibers from the myoglobin stained light micrographs were all identified as having a dark, intermediate or a light reaction. This was recorded directly on the light micrographs.

The last step to this examination involved a cross-comparison of the same fibers throughout all the reactions (Plate 5). The investigator proceeded to go through the ATPase, NADH-TR and myoglobin reaction, one fiber at a time. A list was made of each fiber and the classification of fiber type it received in each reaction. After some experience it was not difficult to identify the same fiber throughout all reactions, despite obvious swelling from the freezing artifact, in the myoglobin stained fibers. A total of 842 fibers were identified as either type I, IIA or IIB. 99.1% of fibers classified as type I stained darkly in myoglobin. 94.8% of those classified as type IIB stained lightly in myoglobin, and 80.3% of type IIA fibers stained intermediately in myoglobin (See Table A). The NADH-TR stain showed reactions somewhat the same as the myoglobin stain, but the oxidative spectrum was too diverse to be used for fiber identification.

It was concluded from this study, that the myoglobin reaction is a valid technique for identification of fiber types in glutaraldehyde fixed tissue.



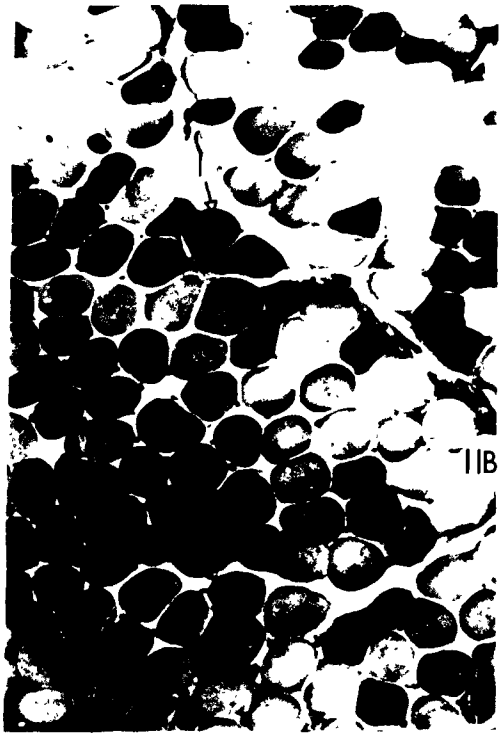
M-ATPase 10.0



M-ATPase 4.6



NADH-TR



Myoglobin

PLATE 5

Table A

Number of Fibers Stained for Myoglobin

	Fiber Type		
	I	IIA	IIB
Total Fibers	420	106	316
Myoglobin Staining Intensity			
Strong	416	1	0
Intermediate	4	85	16
Weak	0	20	300
Accuracy of Myoglobin for Fiber Identification	99.1%	80.3%	94.8%

Appendix III

Mean Myofibrillar Volume Density % $\bar{X}(\pm SD)$

Subject	Type I	Type II	t ratio	p
DF	78.39 ($\pm$5.54)	80.53 ($\pm$6.54)	1.69	$<.10$
PG	80.07 ($\pm$4.98)	82.7 ($\pm$5.65)	4.27	$<.01$
GB	82.29 ($\pm$5.48)	84.26 ($\pm$5.49)	1.94	$<.10$
TC	81.78 ($\pm$5.27)	82.37 ($\pm$4.99)	1.00	$<.20$
LC	81.11 ($\pm$5.26)	82.49 ($\pm$4.26)	2.35	$<.05$

Appendix IV

Mean Mitochondrial Volume Density %(±SD)

Subject	Type I	Type II	t*
DF	6.58 (±4.21)	2.38 (±1.51)	9.62
PG	6.73 (±3.17)	1.60 (±1.17)	18.60
GB	4.52 (±3.38)	1.62 (±1.59)	11.60
TC	5.01 (±3.41)	1.08 (±1.15)	11.35
LC	6.06 (±4.46)	1.12 (±1.90)	9.35

*all t values, $p < .001$

Appendix V

Ratio of Mitochondrial/Myofibrillar Volume Densities $X10^{-2}$

Subject	Type I	Type II	t+
DF	8.39 (±4.85)	2.96 (±1.45)	13.14
PG	8.41 (±4.07)	1.94 (±1.56)	18.17
GB	5.49 (±3.22)	1.14 (±1.84)	14.65
TC	6.12 (±4.14)	1.31 (±1.20)	13.66
LC	7.47 (±4.2)	1.36 (±1.31)	17.01

+$p < .001$

Appendix VI

Mean Cytoplasm Volume Density %(±SD)

Subject	Type I	Type II	t ratio
DF	10.59 (±4.85)	10.91 (±5.10)	1.24^{+}
PG	8.88 (±6.66)	8.76 (±4.52)	$.258^{++}$
GB	8.85 (±4.22)	9.33 (±5.04)	1.44^{+}
TC	8.46 (±4.39)	9.03 (±5.00)	1.04^{+}
LC	10.66 (±4.66)	9.74 (±4.87)	0.602^{++}

$^{+}p < .20$

$^{++}p > .20$

Appendix VII

Mean Lipid Volume Density % (±SD)

Subject	Type I (%)	Type II (%)	t ratio
DF	1.87 (±.34)	.0388 (±.211)	8.69^{+}
PG	1.71 (±.189)	.042 (±.031)	71.3^{+}
GB	.458(±.157)	.0138 (±.08)	3.5^{+}
TC	.644(±.155)	.0452 (±.013)	3.31^{+}
LC	.222(±.90)	.048 (±.312)	2.22^{-}

$^{-}p < .02$

$^{+}p < .001$

Appendix VIII

Mean Volume Density of Sarcoplasmic Reticulum %(±SD)

Subject	Type I	Type II	t++
DF	2.94 (±3.12)	6.14 (±4.28)	10.67
PG	2.86 (±2.08)	5.86 (±3.22)	9.58
GB	3.82 (±2.06)	6.49 (±3.02)	15.3
TC	2.74 (±1.70)	6.67 (±3.55)	11.21
LC	2.57 (±1.94)	6.14 (±3.33)	11.31

++ all t values, $p < .001$

Appendix IX

Ratio of Mean Sarcoplasmic Reticulum/Myofibrillar Volume Densities $X10^{-2}$

	Type I	Type II	t*
Subject			
DF	3.75 (±2.7)	7.62 (±5.31)	7.95
PG	3.57 (±2.59)	7.09 (±3.89)	9.22
GB	4.65 (±3.28)	7.70 (±3.58)	7.69
TC	3.34 (±2.07)	8.09 (±4.31)	12.17
LC	3.14 (±2.39)	7.44 (±4.04)	11.21

* $p < .001$

Appendix X

Mean Fiber Areas X100 ^{2}

Subject	Type I	Type II	t
DF	37.3 (±9.7)	44.75 (±9.13)	3.95$^{\cdot}$
PG	69.14 (±10.39)	86.67 (±15.05)	6.77$^{\cdot}$
GB	50.15(±14.51)	58.57 (±15.65)	2.78$^{\cdot}$
TC	78.82 (±23.33)	84.51 (±28.3)	1.09$^{\cdot\cdot}$
LC	63.96 (± 25.17)	74.7 (±22.56)	2.24^{+}

$^{\cdot}p < .01$

$^{+}p < .05$

$^{\cdot\cdot}p < .30$

Appendix XI

Mean Myofilament Packing Density Number (±SD)/.5μ^2

Subject	Type I	Type II	t
DF	139.7 (±9.45)	135.1 (±13.23)	0.123
PG	143.66 (±19.26)	144.0 (±7.34)	0.237
GB	138.0 (±12.92)	138.9 (±11.48)	0.702
TC	144.83 (±8.73)	144.73 (±18.66)	0.519
LC	135.73 (±12.01)	141.05 (±12.83)	0.249

1971). Further studies on summation and tetanus have shown that with each stimulus given, more calcium was released by the SR (Kirby et al, 1975; Taylor et al, 1975). This corresponded to increased tension outputs, until tetanus was achieved. However the previous authors found that during tetanus when force production levels plateau, calcium is still released by the SR. This suggests that there may be a saturation point for calcium, and when it exceeds a certain concentration, no further increases in force are possible.

If a greater Vv_{sr} does allow a greater absolute calcium release and thus have a greater tension produced per unit area, then a smaller Vv_{sr} would release less total free calcium (decreased calcium concentration) and therefore produce less tension. This may in fact be the case, since Endo (1972), observed that tension decreased from 40% to zero over a two-fold decrease of free calcium.

Greater total calcium released from SR of type II fibers (because of greater Vv_{sr}) may also account for the faster twitch contractions time observed in type II fibers. Twitch contraction time differences have often been attributed to the M-ATPase concentration in each fiber type (Bárány, 1967). However, it has been shown in skinned frog fibers that the rate of fiber shortening is dependent upon the free calcium concentration (Julian, 1971; Thames and Podolsky, 1973). In addition to shorter time to peak tension (Burke and Edgerton, 1975), type II fibers also have shorter relaxation times. One might speculate that the greater surface area of SR found in type II fibers would result in greater rate of release and total uptake of free calcium after depolarization has ended. Type II fibers have previously been observed to have greater rates of

calcium release and greater total calcium accumulation than type I fibers (Fiehn and Peter, 1971; Van Winkle et al, 1978b). Calcium uptake has been closely correlated to relaxation time (Fiehn and Peter, 1971). It is also known that the total SR surface membrane is responsible for calcium uptake (Ebashi, 1976), therefore, type II fibers have the ability for a greater rate of calcium uptake. Greater Vv_{sr} in type II fibers could then account for both a greater tension production per unit area (because of greater calcium release) and the faster twitch contraction times (because of greater calcium release and a greater SR membrane surface area for re-uptake of calcium) found in these fibers.

It might be argued that differences in SR surface area alone, will account for these physiological differences. However, this argument fails to account for the greater tensions per unit cross sectional area produced in type II fibers. As previously mentioned, Vv_{myof} was found to be greater in type II fibers. This would result in a greater quantity of contractile protein per unit cross sectional area, therefore a potential for greater tension production. It may be that the quantity of calcium ions released from SR under normal twitch contraction conditions (excluding summation or tetanus) are similar between fiber types. Tension differences may be a function of Vv_{myof}. However, since it is known that calcium release and uptake is greater in animal FT muscle, it must be assumed that the quantity of calcium released also influences the tension production. Further quantitative studies of calcium release during contraction of human type I and II fibers will clarify the role of calcium in tension production.

A final possibility is that the greater distribution of Sr in type II fibers (and more specifically the Vv_{sr}/Vv_{myof} ratio), may shorten

contraction time simply by decreasing the diffusion distance for calcium, between the SR membrane and the contractile protein.

4.VIE Relationship between Hypertrophy and Sarcoplasmic Reticulum Volume Density

Muscle fiber hypertrophy may be growth induced or training induced. The condition of training induced hypertrophy will be briefly discussed. The question may be asked whether or not SR volume density may be expected to change with increases in fiber size? Hypertrophy of EDL and SOL muscles of the rat achieved by the removal of the synergist muscle was found to have an associated decrease in twitch contraction speed (Lesch et al, 1968; Gutmann et al, 1971). Muscle hypertrophy which was induced by weight lifting activities in cats have also shown a significant decrease in speed of muscle contraction (Gonyea and Bonde Petersen, 1978). A similar decreased twitch contraction time was found in humans who achieved muscle hypertrophy by means of heavy resistance training (Sale, 1980; personal communication).

With training induced hypertrophy, the quantity of SR might be expected to increase proportional to the increase in contractile protein. If this were not the case, the ratio of SR to contractile protein would decrease. This would result in a slower twitch contraction time, since the rate of calcium release and uptake would be decreased. This may account for the findings of the previously mentioned authors.

4.VIIA Fiber Areas

When all subjects were treated as a single group, type II fibers were found to be approximately 16.7% larger ($p < .01$) than type I fibers (69.84 vs. 59.87 x 10^2 respectively). Although type II fiber areas are reported to be larger in most muscles, the vastus lateralis has often been

observed to have similar fiber areas with that of type I fibers (Edström and Nyström, 1969; Cullen and Weightman, 1975).

The present data showed that while there are considerable differences between the area measurements of some subjects within type I and II fibers (coefficient of variance was 27.76 and 25.97% respectively), subjects with large type I areas also had large type II areas. The type II fiber areas found in this study were slightly greater than found by Edström and Nyström (1969). The larger type II fibers observed in this present study may be a function of the types of activities which the subjects pursued in their physical education curriculum. While none of the subjects were trained for any particular sport or activity, subjects PG and TC admitted to performing occasional weight training exercises. A preferential hypertrophy of type II fibers in heavy resistance training (MacDougall et al, 1979) may account for increased type II areas in some subjects.

4.VIIB Differences in Fiber Area

Muscles such as the triceps brachii or biceps brachii have considerably larger type II fibers than type I fibers (Edstrom and Nystrom, 1969; MacDougall et al, 1980), but the fiber areas of the vastus lateralis are reported very similar (Edström and Nyström, 1969; Ingjer, 1977). The fiber areas may reflect the functional differences between different muscles. Normal day to day activities recruit the muscles of the upper arm very little while in the legs, fibers are regularly recruited in normal activity, thereby leading to trained type I fibers (MacDougall et al, 1980). Apparently, this was not the case in the present study since the difference in fiber areas were significant ($p < .01$).

4.VIII Myofilament Packing Density

When the subjects were regarded as a single group, the myofilament packing densities were similar ($p > .10$) for type I and II fibers (140.38 and 140.76/.5 ^{2} respectively).

The only previous report of myosin packing densities is that given by Penman (1969). The results of the present study are somewhat greater than those reported by Penman (1969), however the differencss may be methodological. Since packing density is directly proportional to the sarcomere length (Brandt et al, 1967), the tissue of this study may have had greater contracture after excising or during fixation than Penman's (1969) tissue samples. Even if the muscle samples from this study did have greater contraction, the comparison of type I and II fibers are still valid since both fiber types have undergone identical preparations and therefore have the same degree of contracture.

As already mentioned, type II fibers have greater tension output per cross sectional area. This tension is not accounted for by the number of myosin filaments per unit area. Therefore, one must conclude that the greater tension per unit cross sectional area is due to greater Vv_{myof} and greater calcium concentration (due to greater Vv_{sr}) per unit of contractile protein.

The reasons for constant myosin packing densities between fiber types are not known. It could be speculated that if the myosin filaments were closer together the length of the cross bridge and/or the angle of cross bridge attachment would change. Cross bridge changes must also occur with a decreased packing density. It appears that there is a particular length and angle of cross bridge interaction that is optimal,

and any deviation of this is not practical for contraction purposes. Therefore, if greater tension must be developed (e.g. heavy resistance training) the muscle fiber will add new filaments, but will not decrease the distance between myosin (and thus actin and myosin). For this reason hypertrophy of the fiber must occur (i.e. the fiber must add filaments to the periphery of the sarcomere, thus increasing the sarcomere and therefore fiber size), and the fiber is not able to add new filaments within the existing structure, since the cross bridge lengths and/or angle would be changed and adversely affect muscle contraction.

4.IX Subtypes of Type II Fibers

This study has limited the discussion to the grouped type II fibers. However, it is known that type II fibers can be further subdivided into two major divisions (type IIA and IIB fibers) based upon histochemistry, biochemistry and longitudinal ultrastructural techniques.

The myoglobin localization method which was used for this present study was able to differentiate between type IIA and IIB fibers. However, the tissue which was cut on the EM cryostat only allowed a 2.0μm thick section to be cut for fiber identification. These sections proved to be too thin for differentiation between fiber subtypes, but did allow identification of the larger type I and II groups.

In an attempt to determine possible ultrastructural differences between IIA and IIB fibers, frequency histograms were compiled for the group of subjects for Vv_{mit} and Vv_{sr}. The Vv_{mit} histogram revealed a clear positively skewed curve, in which a large number of fibers had a very low Vv_{mit}, with a wide range and some fibers having very high Vv_{mit} values (see Figure 4.1). Type IIB fibers are known to have a lower

oxidative capacity and would therefore be expected to have a lower Vv_{mit}. Thus presumably, those type II fibers which had very low Vv_{mit} might be assumed as being type IIB, and those with high Vv_{mit} values might be assumed to be IIA fibers. The configuration of the histogram (p. 113) indicates that a clear subdivision between fiber subtypes was not possible and that there is a wide overlap for Vv_{mit} values between type II subtypes.

Animal studies have shown clear differences in mean twitch contraction times between IIA and IIB motor units, but there is a large overlap in the range of these units. The Vv_{sr} frequency histogram for all subjects represented a complex triphasic curve (see Figure 4.2). It is difficult to determine whether these were true curves or if this represented the sample that was selected. A repeat of this study will answer this question.

The SR content covers a large spectrum and as a result it was not possible to differentiate between fiber subtypes from Vv_{sr}. The range of Vv_{sr} would explain the range of twitch contraction times which has been found in type II fibers. It is interesting to note that the Vv_{sr} and Vv_{mit} frequency histograms do not correspond very closely. It would appear that while the range of Vv_{sr} is as large as Vv_{mit}, there are a greater number of fibers with similar Vv_{sr} content. This would suggest that some type II fibers have both high Vv_{mit} (oxidative capacity) and high Vv_{sr} (short twitch contraction time). Such fibers (probably type IIA) are therefore able to do aerobic work and also possess fast twitch contraction times. These volume density measures give further ultrastructural evidence for a fiber subtype (IIA) but the quantity of each volume measure for this fiber subtype is unclear.

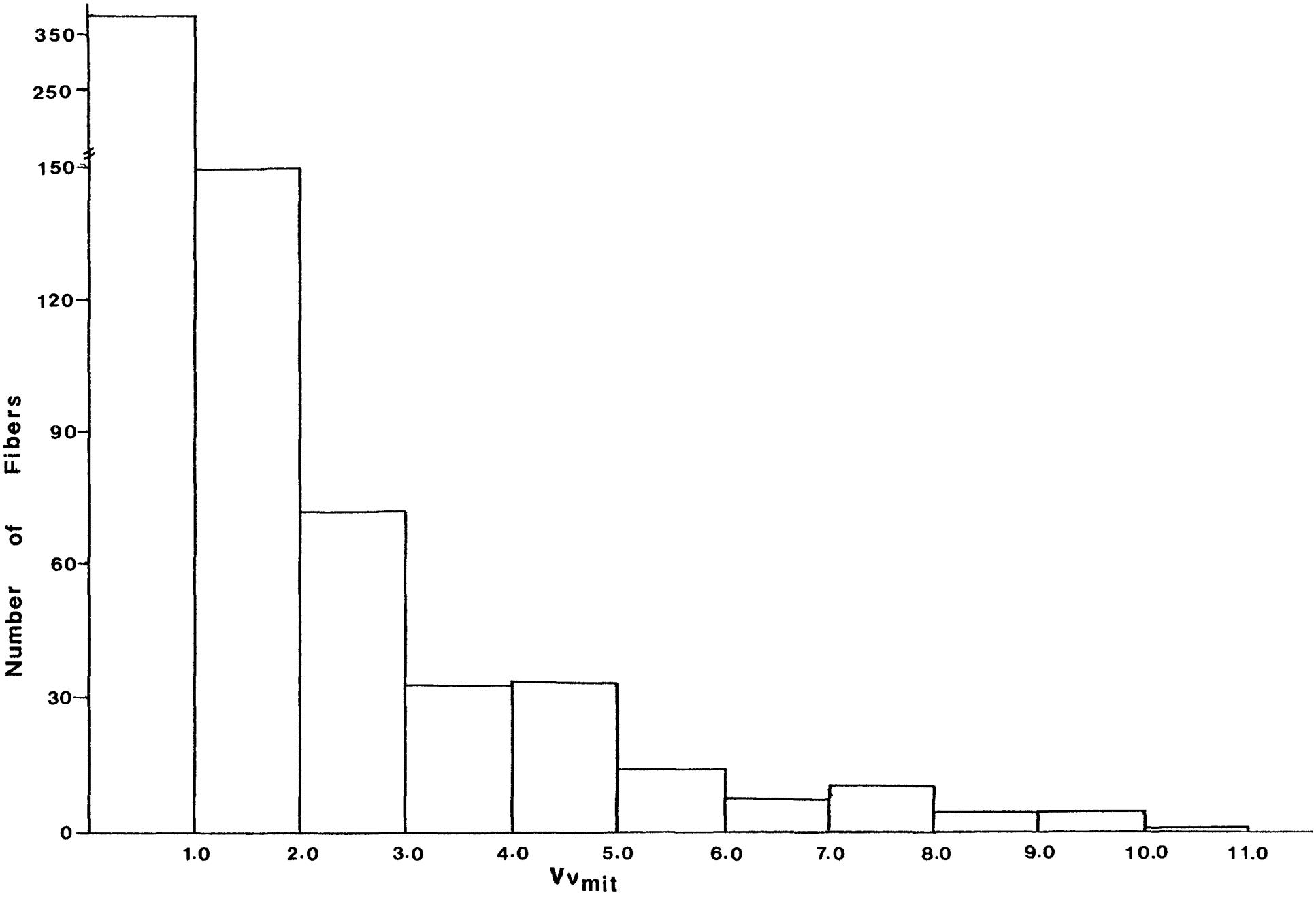


Figure 4.1 MITOCHONDRIAL FREQUENCY HISTOGRAM FOR TYPE II FIBERS

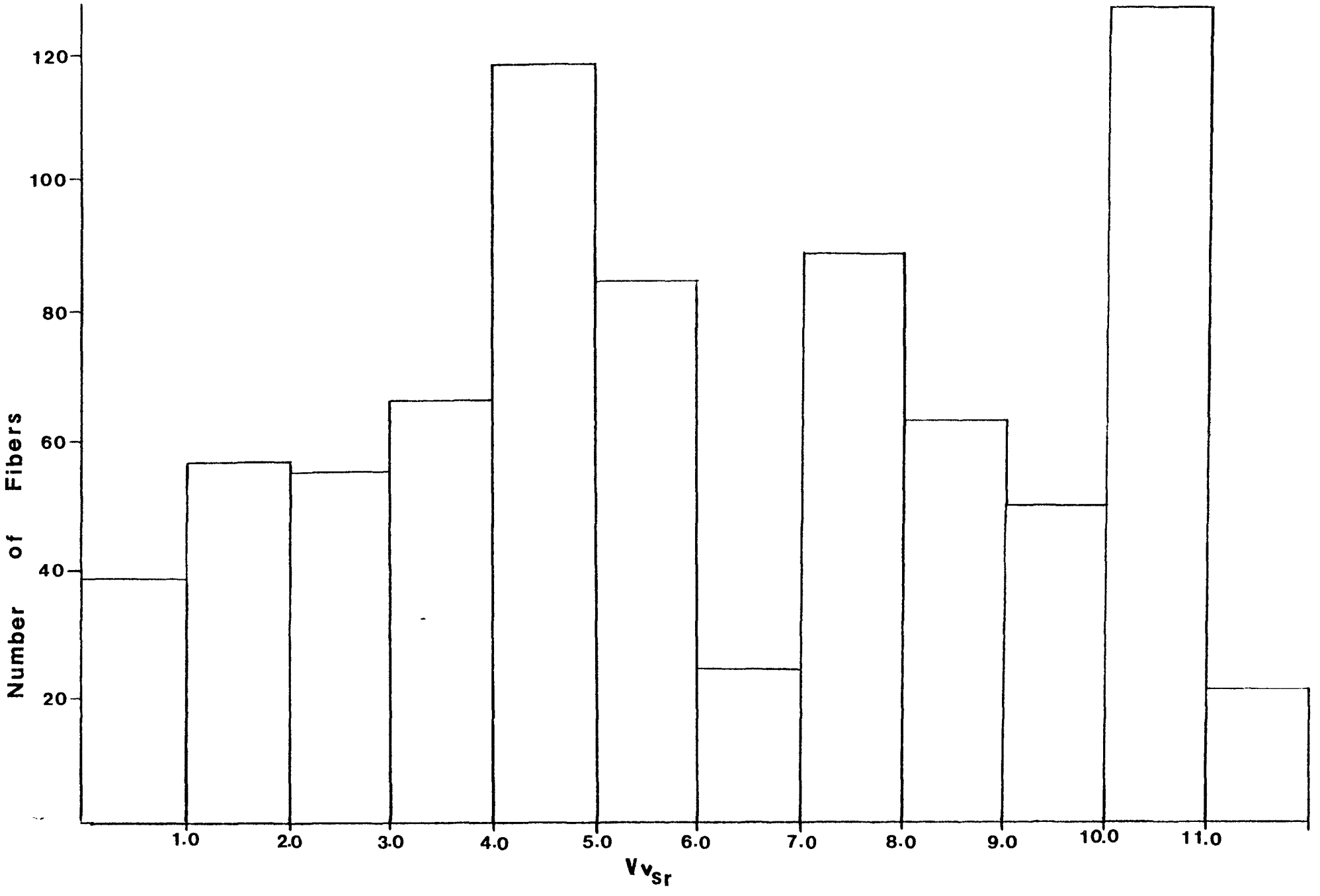


Figure 4.2 SARCOPLASMIC RETICULUM FREQUENCY HISTOGRAM FOR TYPE II FIBERS

4.XA Summary

Accurate identification of fiber types at the ultrastructural level has not previously been accomplished. This study used a myoglobin staining technique for fiber typing which allowed stereological investigation of type I and II fibers in the vastus lateralis. Morphometric analysis showed the following:

1. Vv_{mit}, Vv_{mit}/Vv_{myof}, and Vv_{lip} were greater in type I fibers.
2. Vv_{sr}, Vv_{sr}/Vv_{myof} and Vv_{myof} were greater in type II fibers
3. Vv_{cyt} and myosin packing density were found to be similar between type I and II fibers.

4.XB Conclusions

(1) Greater Vv_{mit} and Vv_{mit}/Vv_{myof} may be one of the factors which account for the greater fatigue resistance of type I fibers.

(2) Greater Vv_{sr} and Vv_{sr}/Vv_{myof} in type II fibers may account for a greater rate of calcium release and uptake and presumably shorter diffusion distances between SR and the contractile protein, thereby allowing faster twitch contraction times in type II than type I fibers.

(3) Greater tension per unit area in type II fibers may be a function of the greater myofibrillar volume density.

Recommendations

The technique for EM preparation of tissue stained for myoglobin should be modified to permit thicker sections and thus positive sub-typing of the type II fibers. The technique would then allow a more quantitative study of diseased muscle, and will be applicable in the study of developing muscle and muscle which has undergone a training stimulus.

Finally, elegant studies which are able to manipulate the amount of calcium released and the amount of active SR from each fiber type may give greater insight into the role of SR in determining twitch contraction times.

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