SKELETAL MUSCLE FIBRE TYPING IN FEMALE ATHLETES:
RELATIONSHIP TO SPECIFIC MEASURES OF PERFORMANCE

A Thesis
Presented to the Faculty of Graduate Studies,
University of Manitoba, in Partial Fulfillment
of the Requirements for the Degree of
Master of Sciences

by
Enid Ruth Brown

December 1979
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ACKNOWLEDGEMENTS

I wish to sincerely thank my supervisor, Dr. F.B. Bertalanffy, for his help and encouragement, without which this project would not have been possible. I would also like to acknowledge Dr. W.J. Dahlgren for her guidance and advice throughout this project.

I wish to express my gratitude to Dr. T.V.N. Persaud for the opportunity to study in this department and for financial support of this research.

To the 'volunteers' from the Anatomy Department, and to the women of the University of Manitoba field hockey and volleyball teams, a heartfelt thank you.

A special thank you to the academic and technical staff of the department for their untiring patience and willingness to help; to Dr. J. Thliveris for the kind use of his lab; and to two very special friends.

I gratefully acknowledge Dr. G. Wilson and Dr. W. Hildahl for their time and skill in performing the biopsies.

I wish to thank Mr. Glen Reid for the graphic illustrations, and Mr. Roy Simpson for the photographs.

Finally, I wish to thank the University of Manitoba for a Graduate Fellowship awarded to me during the tenure of the project.
ABSTRACT

Percentage distributions of type I and type II skeletal muscle fibres in male athletes were previously related to success in a variety of sports. However, little information exists relating specific performance measures to fibre type content, especially in females.

Tests were selected to measure three performance parameters as they relate to the knee extensors. A maximal isometric extension of the knee was used to evaluate isometric strength. Fatiguing extension repetitions with a light weight measured muscular endurance, while power was assessed by a Sargent (vertical) jump.

Muscle samples were obtained by punch biopsy from the right vastus lateralis of eight female field hockey players and four female volleyball players. Tissue samples were snap frozen and serially sectioned at 10 μm. Type I and type II fibre content was determined histochemically using the myosin ATPase technique (pH 9.4).

Regression line correlations did not reveal any significant relationship between the results of the performance tests and size of type I fibres, size of type II fibres, percentage of type II fibres, or percentage area of type II fibres. Comparisons between the volleyball and field hockey players revealed that the field hockey players had significantly larger type I fibres and a significantly smaller percentage of type II fibre area.

Differences in fibre type size and distribution could not be related to performance.
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INTRODUCTION
INTRODUCTION

Fibre type distributions differ among individuals and also between the muscle of one individual. Biopsy studies have revealed that elite* athletes tend to have fibre type distributions characteristic of the demands of their specific sport. It has also been shown that, in some cases, specific forms of training can cause selective hypertrophy of one fibre type. With few exceptions, these studies were carried out with male subjects.

To date, there has been little published that relates fibre type distribution with specific performance parameters. For this reason, it was decided to investigate the possibility of a correlation existing between objective performance test scores and fibre type distribution. The parameters chosen were muscular endurance, muscular strength, and power.

Because of the paucity of published information regarding fibre type distribution in females, it was decided to use only female subjects.

Subjects were selected from intercollegiate volleyball and field hockey teams. Because of this, it was also possible to investigate subgroup differences that related to specific training demands and fibre type distributions.

* For the purpose of this study, 'elite' will refer to those athletes competing at the national or international levels.
REVIEW OF LITERATURE
REVIEW OF LITERATURE

The ability of skeletal muscle to contract upon stimulation is reflected in its highly organized cellular components. The sarcomere is the functional and morphological unit of contraction. Myofilaments are arranged in an interdigitating pattern and are assumed to slide past each other during contraction. It is generally accepted that the thick filaments are responsible for pulling the thin filaments via a cyclic attachment of cross-bridges. The cross-bridges are established by the heads of the myosin molecules.

These myosin heads have an enzymatic function, they cleave the terminal phosphate from ATP molecules. This in turn provides the necessary energy for contraction.

2.1. Substructure of the Myosin Molecule

Thick filaments are chiefly composed of myosin molecules. Each myosin molecule consists of two large subunits called heavy chains and four smaller subunits, the light chains. The two heavy chains are arranged in a helical fashion and form the 'backbone' of the myosin molecule. The light chains form the globular head of the myosin molecule. These light chains show heterogeneity between fast and slow myosins.

Fast muscle myosin has two types of light chains; the DTNB chains, so named because they are removed by 5,5'-dithiobis (2-nitrobenzoic acid), and the alkali chains, dissociated at high pH. The alkali light chains are further divided into a large A1 and a
smaller A2 chain.

Slow muscle myosin has two light chains. One is similar to the DTNB chains of fast myosin, and the other to the alkali light chains.

2.2. Contraction of Skeletal Muscle and ATPase Activity

To date the most widely accepted theory of muscle contraction is the sliding filament theory, proposed by Huxley and Hanson (1960). Variations in the speed of contractions were explained on a basis of cycling frequency of the myosin heads. That frequency, in turn, is dependent upon the ATPase activity of the myosin light chains (Barany 1967). Recent biochemical and immunochemical investigations revealed that there are fast and slow muscle myosins. The physiological differences between muscles, and individual muscle fibres, are a reflection of differences in light meromyosin as well as light chain composition.

2.3. Skeletal Muscle Fibre Types

2.3.1. Classification Systems

It has been known for many years that skeletal muscle fibres were not a homogeneous group. Muscle fibres differ individually in morphology, physiological responses, and enzyme content (Brooke and Kaiser 1970, Burke et al. 1973, Finol 1978). As a consequence, classification of muscle fibres by these parameter has become important both clinically and experimentally.
Gross inspection of whole muscles from various animal species reveals two basic types of fibres. These have been referred to as red and white, dark and pale, or dark and light (Ranvier 1873, Yellin 1967, Gertler and Robbins 1978). Physiological studies of these two types of muscle fibres revealed that, in general, white muscle had a faster twitch time and developed more tension than red muscle (Ranvier 1873, Denny-Brown 1929). On the other hand, red muscle tended to be more resistant to fatigue (Edstrom and Kugelberg 1968, Clamann and Broecker 1979).

Ultrastructural studies also confirmed morphological differences between red and white muscles. Red muscle fibres tend to be smaller in diameter, contain more and larger mitochondria, and have thicker Z-lines (Padykula and Gauthier 1970, Gauthier 1969, Finol 1978). These morphological characteristics have a direct bearing on the physiological and enzymatic profiles of the different fibre types.

Because muscle fibres do not all contract at a uniform speed, or generate uniform tension, it is also feasible to classify fibres according to their physiological responses. The simplest system recognizes two fibre types: fast-twitch (FT) and slow-twitch (ST) (Eberstein and Goodgold 1968, Edgerton et al. 1975, Thorstensson 1976). The FT fibres are analogous to white fibres whereas the ST fibres correspond to the red fibres. All fibres belonging to a single motor unit have the same physiological-morphological characteristics, that is, they are composed entirely of either FT or ST.
fibres (Andersen and Sears 1964).

The field of histochemistry provided the most extensive research in muscle fibre typing, focusing on the enzymes of glycolytic and oxidative metabolism (see Khan 1976 for review). Stein and Padykula (1962) proposed a classification system based on the qualitative differences in succinic dehydrogenase (SDH) staining. They grouped the fibres into A, B, and C categories depending upon their staining intensity. Romanul (1964) suggested that by employing a battery of histochemical techniques, skeletal muscle fibres could be subdivided into as many as eight groups.

Histochemical methods for demonstrating ATPase activity were described by Padykula and Herman in 1955. Since that time a number of classification systems were proposed based on the myosin ATPase activity of muscle fibres.

Yellin and Guth (1970) proposed a system based exclusively on ATPase activity. Fibres designated α were observed to be acid labile, β fibres were base labile, whereas αβ fibres were intermediate. Another classification based on pH lability of the myosin ATPase reaction was introduced by Brooke and Kaiser (1970). This system divided the fibres into two broad groups, type I and type II, dependent on the routine calcium reaction for ATPase (pH 9.4). Type I fibres, being alkaline labile, stained poorly; type II fibres being alkaline stable, stained intensely black. The type II fibres were further subdivided into IIA, IIB, and IIC categories based on
their susceptibility to acid pH. Type IIA were inhibited below a pH of 4.5, type IIB were inhibited below a pH of 4.3, and IIC fibres were inhibited below a pH of 3.9.

Other investigators employed the myosin ATPase staining technique in combination with histochemical reactions for oxidative and/or glycolytic pathway enzymes to classify skeletal muscle fibres. For example, Khan et al. (1973) utilized staining of SDH, routine myosin ATPase (pH 9.4), sarcoplasmic reticular ATPase (SR-ATPase), and creatine kinase (CK) to demonstrate type I and type II fibres in rabbit muscle. Type II white fibres revealed low SDH activity but high myosin ATPase, SR-ATPase, and CK activities. Type II red fibres exhibited high levels of activity of all these enzymes, while type I red fibres had high SDH and CK, but low myosin ATPase and SR-ATPase activities. On the other hand, Dubowitz and Pearse (1960) favored a more simple system of typing. According to their classification type I fibres were high in oxidative enzymes and low in phosphorylase and routine ATPase activity, whereas type II fibres revealed a low reactivity for oxidative enzymes but prominent staining for phosphorylase and routine ATPase activity.

Metabolic and physiologic profiles of muscle fibres led to a correlation between the two. On fourteen different animal muscles, Barany (1967) demonstrated that biochemical assays of ATPase activity correlated well with the speed of muscle shortening; as ATPase activity increased the contraction time decreased. It was now established that the myosin ATPase activity, as it is determined
biochemically, could be related to the histochemical fibre types classified by the ATPase staining reaction.

Essen et al. (1975), employing the routine myosin ATPase technique on human skeletal muscle, demonstrated that type II fibres had approximately two and one half times higher ATPase activity than type I fibres. As a result of these studies, type I and type II fibres are now physiologically classified as ST and FT respectively.

Utilizing the foregoing criteria, Barnard et al. (1971) proposed a classification system based upon the staining reactions for myosin ATPase and NADH-diaphorase. They suggested the existence of three fibre types: fast-twitch red (high routine myosin ATPase and NADH activity), fast-twitch white (high ATPase activity, low NADH activity), and slow-twitch intermediate (low ATPase activity, high NADH activity).

Peter et al. (1972) proposed a system of nomenclature comparable to that of Barnard et al., yet based not on morphology but rather upon biochemical evidence. Studies were performed on guinea pig and rabbit muscles composed predominantly of a single fibre type. Eight enzymes of the glycolytic and oxidative pathways, as well as myosin ATPase activity and glycogen and myoglobin contents were analyzed. Three fibre types were described: fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG), and slow-twitch oxidative (SO).

These observations corresponded, respectively to the fast-twitch white, fast-twitch red, and slow-twitch intermediate fibres of the Barnard et al. classification, and to the type IIB, IIA, I fibres of the system developed by Brooke and Kaiser.
In summary, in more recent studies, most investigators employed a two or three fibre classification system. The two fibre system is based on the routine myosin ATPase staining reaction. Fibres are classified as ST or type I if they exhibit low activity. In human muscle the metabolism of these fibres is chiefly oxidative. Type II fibres or FT fibres are characterized by high myosin ATPase activity (pH 9.4). Metabolism may vary from predominantly oxidative to predominantly glycolytic.

The most notable three fibre classification is the FG, FOG, SO system of Peter et al., or the corresponding I, IIA, IIB of Brooke and Kaiser; type IIC is often ignored because it comprises less than five percent of the normal population of human muscle and is thought to be an undeveloped or transitional muscle fibre (Brooke and Kaiser 1970, Jansson et al. 1978).
Summary of two- and three-fibre classification systems.

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<tr>
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<th>Reactivity for Oxidative Enzymes</th>
<th>Reactivity for Glycolytic Enzymes</th>
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<tbody>
<tr>
<td>ST</td>
<td>low</td>
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<tr>
<td>Type I</td>
<td>low</td>
<td>high</td>
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<tr>
<td>FT</td>
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<td>moderate to high</td>
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<tr>
<td>Type II</td>
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<td>low to high</td>
<td>moderate to high</td>
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<td>SO</td>
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<td>low</td>
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<tr>
<td>Type I</td>
<td>low</td>
<td>high</td>
<td>low</td>
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<tr>
<td>FOG</td>
<td>high</td>
<td>moderate to high</td>
<td>moderate</td>
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<tr>
<td>Type IIA</td>
<td>high</td>
<td>moderate to high</td>
<td>moderate</td>
</tr>
<tr>
<td>FG</td>
<td>high</td>
<td>low</td>
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<td>Type IIB</td>
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2.3.2. Innervation and Convertibility

It has now been established that the two basic muscle fibre types are supplied by different types of neurons. Slow muscle fibres are innervated by tonic alpha motoneurons, fast fibres by phasic alpha motoneurons. Compared to phasic neurons, tonic neurons have smaller cell bodies and axons of a smaller calibre (Granit et al. 1956, Eccles et al. 1958). Tonic neurons also have a lower threshold value, a slower conduction velocity, and a longer after-hyperpolarization duration (Eccles et al. 1958). These observations indicate that the type of innervation a muscle fibre receives determines its intrinsic speed of contraction. Cross-innervation studies in muscles of kittens and rats revealed that a slow muscle, such as the soleus, could mimic the physiological properties of a fast muscle, such as the extensor digitorum longus (EDL or FDL), with innervation by the nerve to the fast muscle (Buller et al. 1960, Buller et al. 1969, Barany and Close 1971).

Using the cross-innervation model, Buller et al. (1960) concluded that a substance(s) passed from the neuron to the muscle fibre thereby establishing and maintaining the muscle's contractile properties. Since then, doubt has been cast on this theory when it was discovered that fast muscle could mimic the properties of slow muscle through direct electrical stimulation of the intact nerve, at frequencies of ten impulses per second for various time periods (Salmons and Vrbova 1969, Rubinstein et al. 1978). Further cross-
innervation studies revealed that the critical site of neuronal influence was in fact the ATPase activity of myosin (Barany and Close 1971).

2.3.3. Development of Skeletal Muscle Fibre Types

Human skeletal muscle in its early stages of embryonic development is composed of a homogeneous population. Until twenty weeks gestation there are no distinct histochemical differences among the fibres. By twenty-two weeks type I and II fibres can be discerned with the routine myosin ATPase technique, while type IIB and IIC fibres are present in the neonate (Ringqvist et al. 1977). Studies in rats indicate that type I and IIC fibres predominate initially. Subsequently the proportion of IIC fibres diminishes at the expense of an increase first in type IIB fibres, and then IIA fibres (Brooke et al. 1971).

In its early, undifferentiated stages, fetal muscle is uniformly slow in contracting. However, Gauthier et al. (1978) demonstrated the coexistence of fast and slow myosins within individual fibres of the rat diaphragm. This is probably a consequence of its polyneural innervation. As the incidence of polyneural innervation decreases, fibre typing becomes more definitive, until at about day nineteen, in the rat, all fibres are singly innervated, and the fibre typing characteristics of the adult rat are established (Gauthier et al. 1978).
2.4. Fibre Typing in Athletics

2.4.1. Fibre Type Distribution

Most biopsy studies of athletes and non-athletes utilized the vastus lateralis muscle because of its accessibility in terms of size and superficial location, as well as its involvement in many large muscle activities. In the normal, untrained male, the type I fibre distribution of this muscle has been reported as 44% (Thorstensson et al. 1977), 46% (Thomson et al. 1979), 52% (Taylor et al. 1974), 52.6% (Costill et al. 1976a), and 55.9% (Komi and Karlsson 1978). There is, therefore, a notable variance even within an untrained population. However, it is reasonable to assume the normal distribution in vastus lateralis to be approximately 50% type I fibres and 50% type II fibres plus or minus 5 to 10%.

Many studies of elite athletes indicated a tendency toward a fibre type distribution characteristic of the athlete's particular sport. Studies of elite male athletes competing in highly aerobic activities have shown such athletes to have a predominance of type I fibres. For example, biopsies of orienteers revealed that the average percentage of type I fibres in vastus lateralis was 68% (Jansson and Kajser 1977) and 77% (Thorstensson et al. 1977). Costill et al. (1976a) observed that the average type I fibre content in vastus lateralis of male elite distance runners was 79%.

In contrast, the percentage of type I fibres in elite sprinters, whose metabolic demands are more anaerobic, was reported as 24%
(Costill et al. 1976a) and 39% (Thorstensson et al. 1977).

Although profiles on female subjects are few, most indications are that the distribution of the two fibre types in female non-athletes is also approximately 50:50 (Costill et al. 1976a, Taylor et al. 1978, Komi and Karlsson 1979). Prince et al. (1977), however, reported an average of 36.4% SO fibres in untrained females.

It has been noted that elite female sprinters have an average of 27.4% ST fibres (Costill et al. 1976a) and that intercollegiate field hockey players have a mean SO fibre distribution of 48.2% (Prince et al. 1977). However, studies attempting to relate fibre type distribution in female athletes to success in a particular sport are few.

2.4.2. Fibre Size

Individual fibre size (cross-sectional area) exhibits great variability even within the untrained population. However, within any given (untrained) sample population, type I fibres tend to be smaller than type II fibres. In untrained males, type I fibre size falls within the range 3000 to 6000 \( \mu \text{m}^2 \), while type II fibres range from 3500 to 7500 \( \mu \text{m}^2 \). In trained male athletes, type I fibres may range from 5000 to 9000 \( \mu \text{m}^2 \), while type II fibres range from 5000 to 10,000 \( \mu \text{m}^2 \) (Gollnick et al. 1972, 1973, Thorstensson et al. 1975, Larsson et al. 1978, Ingjer 1979, Costill et al. 1976b, 1979). These ranges are a reflection of interindividual differences rather than intraindividual variability.
Muscle fibres of females tend to be smaller than those of their male counterparts. Although there is little documentation of either the untrained or athletic female, mean type I fibre size in untrained women was reported to be 2784 µm.\(^2\), (Prince et al. 1977) and 3875 µm.\(^2\) (Costill et al. 1976a), while values for FG and FOG fibres were 2425 µm.\(^2\) and 3392 µm.\(^2\) (Prince et al. 1977), and FT fibres were 4193 µm.\(^2\) (Costill et al. 1976a).

In studies comparing untrained and trained females, the differences parallel those observed in the male population, that is, untrained women have smaller fibres than trained women. Most investigations of trained females have shown both FT and ST fibres to have cross-sectional areas of 4000 to 6000 µm.\(^2\) (Costill et al. 1976a, Burke et al. 1977, Prince et al. 1977). However, average values as large as 9003 µm.\(^2\) for ST fibres and 8557 µm.\(^2\) for FT fibres have been reported in endurance trained women (Taylor et al. 1978).

2.4.3. Selectivity of Motor Unit Recruitment

Glycogen depletion studies indicate that fibre types are preferentially recruited according to exercise demands. Type I fibres are heavily recruited during prolonged exercise of lower intensity whereas type II fibres exhibit their greatest involvement in intermittent or continuous activity of maximal intensity (Andersen and Sjogaard 1975, Green 1978). Gollnick et al. (1974) demonstrated a selective glycogen depletion that depended upon the strength of contraction. Type I fibres were recruited for sustained
contractions of 20%, or less, of maximum voluntary contraction (MVC), while type II fibres were recruited for tensions above that level. In studies where three fibre types were distinguished, type IIA fibres were recruited to a greater extent than IIB fibres in submaximal workloads. Of the three, type IIB fibres exhibited the greatest amount of depletion in supramaximal workloads (Andersen and Sjøgaard 1975, Thomson et al. 1979). Only in exhaustive supramaximal exercise did all fibre types display a substantial depletion (Edgerton et al. 1970, Essen 1978).

Differences in fatigability between fibre types has also been demonstrated. Edstrom and Kugelberg (1968) observed in rats, after repeated stimulation of motor units, that the three fibre types differed in fatigue times. Type C showed no fatigue, type B were intermediate in fatigability, while type A fibres fatigued most quickly. A positive correlation ($r = 0.86$) between fatigue and the proportion of type II fibres was found to occur in the vastus lateralis of human subjects (Thorstensson and Karlsson 1976).

Recruitment and fatigability of motor units are most dependent upon strength of contraction, contraction velocity, and intensity of work (Thorstensson and Karlsson 1976, Green 1978, Thomson et al. 1979).

2.4.4. Convertibility of Fibre Types in Humans

Animal experiments have confirmed that muscles can change their fibre type distribution. However, cross-innervation and chronic
stimulation experiments are obviously not feasible in studies using human subjects. Therefore, investigations into the convertibility of human muscle fibres necessarily involves the use of training as the primary stimulus.

Muscle fibre types may show a selective adaptation that is dependent upon the nature of the training stimulus. Strength training programs have been shown to increase the area of FT to ST fibres (Thorstensson et al. 1976a, Costill et al. 1979).

The training program of Thorstensson and coworkers consisted of squat repetitions with a weight corresponding to the subject's 6 repetition maximum (6 RM). Sessions were performed three times per week for eight weeks. This resulted in a significant increase in the ratio of FT to ST area. There was, however, no significant change in either FT or ST area. It is important to note that a negative correlation ($r = -0.62$) was found between percent of FT fibres and percent increase in MVC.

Costill et al. (1979) used an isokinetic training program of leg extensions performed at a velocity of 3.14 rad./s. The training sessions were conducted four times per week for seven weeks. This resulted in significant increases in the percentage of type I and type IIA fibre areas. There was also a significant increase in the percentage area ratios of type IIA to type I and type IIA to IIB. Attempts to relate these changes to gains in strength or fatigability of the muscle during maximal isokinetic contraction were unsuccessful.

Using a training program of squat repetitions at either 50 or
80 percent of 1RM, Dons et al. (1979) observed no change in fibre type percentage distribution, or in FT area/ST area. There was, however, a significant positive correlation \((r = 0.80)\) between increase in dynamic strength relative to muscle cross-sectional area, and percent of FT fibres.

Clearly, different modes of strength training have widely varying effects that are difficult to relate to changes in fibre type distribution.

Endurance training may also affect fibre type composition. Gollnick et al. (1973), using male subjects, showed a significant, selective hypertrophy of ST fibres and an increase in the ratio of ST to FT fibre areas after a five month training program. Training required four, one-hour sessions per week on a bicycle ergometer at 75 to 90 percent of the subject's VO\(_2\)max. (maximal oxygen uptake).

In an investigation of the effects of endurance training on muscle composition of men and women, Taylor et al. (1978) found significant increases in the ST fibre size of both sexes. In addition, there was no indication of an increase in FT area/ST area even though the increase in VO\(_2\)max. (11% for females, 16% for males) was similar to the 13% increase observed by Gollnick et al. (1973).

There is no conclusive evidence indicating that training can cause an actual change in fibre type distribution. Although biochemical and histochemical changes in the enzymes of glycolytic and oxidative metabolism are well documented, there is no substantial proof of changes in fibre type as determined by the myosin ATPase
technique. There is a suggestion that subgroups of type II fibres may undergo change with intensive endurance training. In a study by Andersen and Henriksson (1977) endurance training resulted in a significant increase in the percentage of IIA fibres and a corresponding decrease in IIB fibres. They interpreted this as a gradual conversion of the more glycolytic IIB fibres into the more oxidative type IIA fibres. The training program was eight weeks in length, with sessions four times per week. The subjects were required to pedal a bicycle ergometer for 30 minutes at 80 percent VO₂ max. A significant increase of 18 percent in VO₂ max. was observed.

In an investigation of the effects of anaerobic versus aerobic training, Jansson et al. (1978) reported data that they believed was indicative of fibre type transformation. Runners underwent approximately 18 weeks of aerobic endurance training and 11 weeks of anaerobic training. All subjects showed a decreased percentage of type I fibres and an increased IIC percentage after anaerobic training compared to after aerobic training. An increase in type IIA and IIB percent and IIB/IIA percent was also noted after anaerobic training. These workers hypothesized that the increased IIC fibre population was due to the transformation of type I fibres to type IIA fibres with the IIC fibres representing the transitional stage of change.

It is evident that no conclusive statements can be made regarding the effects of training on fibre type. With respect to strength training, isokinetic and isotonic programs produce varying results.
The effects of endurance training are also variable. Even though increases in \( \text{VO}_2\text{max} \) are comparable among the studies of Gollnick et al. (1973), Taylor et al. (1978), and Andersen and Henriksson (1977), each reported different effects on muscle composition.

2.4.5. Fibre Type and Objective Measures of Performance

Studies characterizing the fibre type distribution of male athletes are numerous. They include middle and long distance runners, sprinters, orienteers, jumpers, throwers, downhill skiers, race walkers, canoeists, hockey players, swimmers, weight lifters, and cyclists (Gollnick et al. 1972, Costill et al. 1976a, Tesch et al. 1976, Jansson and Kaisjer 1977, Prince et al. 1976, Thorstensson et al. 1977, Bergh et al. 1978). In females, only track athletes, field hockey players and cyclists have been studied. Few studies, however, have attempted to relate fibre type distribution to objective measures of performance, such as, \( \text{VO}_2\text{max} \), isometric strength, dynamic (isotonic and isokinetic) strength, vertical jump, anaerobic power, etc.

The most widely used measure of aerobic capacity is \( \text{VO}_2\text{max} \). In a study of elite male and female track athletes Costill et al. (1976a) found no relationship between percent ST fibres and \( \text{VO}_2\text{max} \). Values for \( \text{VO}_2\text{max} \) were obtained with a maximal treadmill test. Burke et al. (1977) also failed to correlate percent ST fibres and \( \text{VO}_2\text{max} \) in a group of male and female cyclists. In this study, \( \text{VO}_2\text{max} \) was established by either riding a bicycle to exhaustion on a graded
treadmill or by performing on a bicycle ergometer until a revolution rate of 60 RPM could not be maintained.

Using either exhaustive treadmill or bicycle ergometer tests, Bergh et al. (1978) noted a positive correlation between percent ST fibres and VO$_2$max. In highly trained athletes competing at the international level, the correlation coefficient was .72; for moderately trained athletes, .34. It is of interest that, although a positive correlation was found, highly trained subjects with the same ST percentage as moderately trained subjects had a higher VO$_2$max.

There are also discrepancies in the literature regarding fibre type and strength measurements. Attempts by Thorstensson et al. (1976b), Hulten et al. (1975), Thorstensson (1976), and Dons et al. (1979) to correlate isometric strength in males with percent FT or ST fibres was unsuccessful. In a study of elite female athletes, Gregor et al. (1979) also found no correlation. These results are in opposition to studies by Komi and Karlsson (1979) and Tesch and Karlsson (1978) who found a significant negative correlation between isometric strength and ST fibre content.

Relationships between fibre type distribution and torque values have recently been investigated using isokinetic devices. Subjects with higher percentages, or percentage areas, of FT fibres show greater peak torque productions. The significance of the relationship between peak torque and FT composition increases with speed of shortening (Thorstensson et al. 1976a, Coyle et al. 1979, Gregor et al. 1979).
Data relating objective measures of power to fibre type are few in number and conflicting. In a study of monozygous and dizygous twins, Komi and Karlsson (1979) found no relationship between power, determined by the anaerobic power test of Margaria et al. (1966), and percent ST fibres. However, Bosco and Komi (1979), did find a significant positive correlation \((r = .51)\), between height of rise of centre of gravity (vertical jump) and percent FT fibres.

When comparing fibre type distribution to performance measures, conflicting results may, in part, be due to differences in testing procedures. Power may be evaluated in many ways. The opposing conclusions reached by Komi and Karlsson (1979) and Bosco and Komi (1979) may be the result of using different methods to measure power. Discrepancies among studies relating ST fibre composition to \(V_O^{2\text{max}}\) may also be the result of different testing methods. The use of maximal versus submaximal tests and a bicycle ergometer as opposed to a treadmill may all influence the values obtained for \(V_O^{2\text{max}}\).
PRELIMINARY INVESTIGATION
PRELIMINARY INVESTIGATION

3.1. Introduction

A preliminary study was undertaken to determine the reproducibility of the myosin ATPase technique on animal as well as human skeletal muscle. Preliminary biopsies were performed to establish the technique of handling the material and the minimum acceptable size of specimen.

3.2. Materials and Methods

3.2.1. Muscle Samples

Three male adult mice and one male adult rat were killed and skeletal muscle was excised from the thigh. Samples were oriented vertically on labelled cork discs (Dubowitz 1973). The samples were covered with embedding medium (Ames OCT Compound, Fisher Scientific) and snap frozen in isopentane cooled to -160 °C with liquid nitrogen. The specimens were stored at -80 °C. Serial frozen sections were cut on an American Optical cryostat. The cork discs were frozen to cryostat chucks with a drop of water. Sections were air dried at room temperature for one hour. The tissue was then stained for the myosin ATPase reaction.

Human skeletal muscle was also obtained for the preliminary study. Two specimens from surgical amputations were processed as described above. Four samples of skeletal muscle were also obtained
from volunteers (two male and two female). A sample of muscle was removed by the punch biopsy technique (Bergstrom 1962), using a Stille biopsy needle.

3.2.2. Staining Reaction Procedure*

3.2.2.1. Histochemical Materials

1. Basic Medium

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>3.96 gm.</td>
</tr>
<tr>
<td>Calcium Chloride</td>
<td>4.20 gm.</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>3.80 gm.</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>1.90 gm.</td>
</tr>
<tr>
<td>Double Distilled Water</td>
<td>Bring to 1000 ml.</td>
</tr>
</tbody>
</table>

Approximately half of the basic medium was adjusted to pH 9.4 while the other half was adjusted to pH 10.3. Adjustments were made with 5N NaOH and 5N HCl.

2. Acid Medium

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Acetate</td>
<td>6.47 gm.</td>
</tr>
<tr>
<td>Potassium Chloride</td>
<td>3.70 gm.</td>
</tr>
<tr>
<td>Double Distilled Water</td>
<td>Bring to 500 ml.</td>
</tr>
</tbody>
</table>

The acid medium was divided into two parts. Glacial acetic acid was used to bring the pH of the solutions to 4.6 and 4.37.

3. Incubation Medium

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>0.017 gm.</td>
</tr>
<tr>
<td>Basic Medium</td>
<td>10 ml.</td>
</tr>
</tbody>
</table>

The incubation medium was adjusted to pH 9.4 with 1N HCl.

* Method courtesy of Dr. H. Green, University of Waterloo. (pers. comm.)
4. 1% Calcium Chloride Solution

Calcium Chloride 10 gm.
Double Distilled Water 1000 ml.

5. Cobalt Chloride Solution

Cobalt Chloride 36.6 gm.
Double Distilled Water Bring to 1000 ml.

6. Ammonium Sulfide Solution

20% Ammonium Sulfide 5 ml.
Double Distilled Water Bring to 100 ml.

3.2.2.2. Histochemical Method

Serial frozen sections were picked up on glass slides and air dried for one hour. Five slides were prepared from each specimen. One slide was placed in each of the following solutions:

Basic Medium at pH 10.3 for 9 minutes at 37 °C
Acid Medium at pH 4.6 for 50 seconds at room temperature
Acid Medium at pH 4.37 for 5 minutes at room temperature

Two slides were not preincubated. The five slides were then washed in Basic Medium (pH 9.4) for one minute. The wash solution was changed once after 30 seconds.

Subsequently the slides were placed in the incubating medium and warmed at 37 °C in a water bath. As a control, one of the unpreincubated slides was incubated without substrate. After 30 minutes the sections were placed in a 1% CaCl$_2$ solution. The CaCl$_2$ was changed three times at intervals of 1, 2, and 3 minutes. Then the slides were placed in CoCl$_2$ solution for 3 minutes. At this point
the slides were rinsed in distilled water and placed in a solution of 1% \((NH_4)_2S\) for one minute. The slides were rinsed again in distilled water. The slides were then coverslipped.

From serial sections, four fibre types should have been discerned as follows:

<table>
<thead>
<tr>
<th>pH</th>
<th>I</th>
<th>IIA</th>
<th>IIB</th>
<th>IIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- Intense staining
- Moderate staining
- Unstained

3.2.3 Human Biopsy Technique

Punch biopsies, using a 5mm. Stille needle, were performed by two licensed physicians.

The skin over the mid-lateral right thigh was initially swabbed with 70% alcohol and iodine. Approximately 1 cc. of 2% xylocaine was injected subcutaneously with a 25 gauge needle to anaesthetize the area. After ensuring that the skin was properly anaesthetized, a deep stab incision was made. The biopsy needle was introduced into the muscle via the incision, and a small sample of muscle obtained (5 - 10 mgm.).
The incision was closed with an adhesive strip dressing. None of the subjects reported any subsequent complications.

The tissue obtained was processed as described in section 3.2.1. All tissue was examined under a dissecting microscope to establish orientation. At this time all fat and extraneous connective tissue was discarded.

3.3. Results

3.3.1. Myosin ATPase Reaction in Animal Skeletal Muscle

A good staining reaction was observed in all samples at pH 10.3, 9.4, and 4.37. Serial sections of each sample were processed on different days with uniform results. The sections preincubated at 4.37 always showed a complete reversal of staining when compared to sections from pH 10.3 and 9.4. The sections preincubated at pH 10.3 showed the same staining pattern as the pH 9.4 sections. At pH 10.3 the type I fibres were virtually unstained, whereas, at pH 9.4 they were moderately stained. In both cases the type II fibres stained intensely black. The control slides were always unstained.

The staining reaction obtained with sections preincubated at pH 4.6 was inconsistent. Frequently it was not possible to differentiate the subtypes of the type II group.

3.3.2. Myosin ATPase Reaction in Human Skeletal Muscle

The results of the staining reaction in human muscle paralleled
those found in the animal study. Fibres could easily be classified as type I or type II from the sections preincubated at pH 10.3, 9.4, and 4.37. It was not possible to subgroup the type II fibres because of the inconsistencies in the pH 4.6 sections.

3.4. Discussion

The histochemical method for myosin ATPase is dependent upon exacting pH values. Values for subgrouping the type II fibres are especially critical because of the extremely small separation between them (Brooke and Kaiser 1970). For this reason the most probable source of error with the pH 4.6 preincubation was pH variation. This was not a problem with extreme acid and alkaline preincubations; however, because the 4.6 pH preincubation is the intermediate pH value in the series, there is a narrower range for success.

3.5. Conclusions

1. The myosin ATPase reaction for pH 10.3, 9.4, and 4.37 was reliable and reproducible.
2. Because of the inconsistency in the pH 4.6 reaction, it was decided to analyze the specimens in the main study for types I and II only.
3. The staining reactions at pH 9.4 and 4.37 were chosen to determine the two fibre types. Alkaline preincubation at 10.3 was not selected because area determination of the type I fibres was more difficult due to their limited stainability.
Section across entire biopsy specimens of human vastus lateralis muscle (myosin ATPase at pH 9.4). Type I fibres are moderately stained. Type II fibres are intensely stained. Note that each section contains a minimum of 150 fibres. (Top x39, Bottom x55)
Cross section of mouse skeletal muscle (myosin ATPase at pH 10.3). Note that the cell borders of the light type I fibres remained somewhat indistinct. (x480)

Cross section of human skeletal muscle (myosin ATPase at pH 9.4). Note that cell borders of both types are distinct. (x360)
Contiguous sections of rat skeletal muscle. Both sections were preincubated at pH 4.6. Note variation in staining reaction. For instance, the arrow indicates a fibre stained darkly in the top photograph that exhibits just moderate staining in the lower illustration. (x400)
MATERIALS AND METHODS
MATERIALS AND METHODS

4.1. Subjects

Fifteen female volunteers, attending university and participating in intercollegiate athletics, were selected for this study. Six of the volunteers were volleyball team members, the other nine were field hockey team members.

4.2. Performance Tests

Twelve volunteers of both sexes underwent three performance tests to determine muscular strength, power, and endurance. The tests were repeated after a two week interval to establish reliability.

Muscular power of the lower limb was determined by a Sargent jump. The subject was asked to make a mark as high as possible on a tape on a wall. Standing one foot from the wall, the subject was then instructed to jump and make a second mark on the tape. This was performed three times, with a short rest period between jumps. The difference between the standing reach height and the jump reach height was recorded as the value (in cm.) of the subject's vertical jump. The average of the three jumps was recorded as the subject's score on that test.

Muscular strength of the knee extensors was determined by a maximal isometric extension, using a Cybex Orthotron. Only the right leg was tested. The orthotron 'up' control was set for zero (maximum resistance) while the 'down' control was set for 10 (least resistance).
The subject sat on the table and the ankle pad was adjusted for leg length so that it contacted the shin just above the ankle. The strap on the ankle was secured around the lower leg so that it was snug. The subject was then instructed to lie back on the table. The axis of the right knee joint was placed so that it was in line with the axis of the lever on the orthotron. The knee was flexed to $90^\circ$. The indicator dial for the right leg was set to zero. At this point the subject was instructed to apply as much force to the bar as possible. The best score on the three trials was recorded as the subject's isometric strength. Scores were recorded in pounds of force and later converted to kilograms of force.

The endurance performance test was performed on an Exergenic quads table. A light weight (4.5 kg.) was attached to the padded shin guard, and the subject's right leg was secured to the guard with a length of cotton bandage. The subject sat upright on the table and was instructed to extend the knee at a rhythmic rate until exhaustion. A metronome was set at 76 beats/minute and the subject was asked to fully extend on every second beat. The rate of extensions was 38 per minute. A stopwatch was used to obtain the time to fatigue. This test was performed only once per testing session. The number of complete extensions performed was recorded as the subject's score.

The three tests were completed in one session. The Sargent jump was performed first, followed by the isometric strength, and the endurance test. At least two minutes rest was allowed between the
vertical jump and the isometric strength test. At least five minutes was allowed between the isometric strength test and the endurance repetitions.

4.3. Biopsy Technique

The procedure followed was as stated in section 3.2.3.

4.4. Staining Reaction Procedure

The procedure followed was as stated in section 3.2.2. The preincubations at pH 10.3 and 4.6 were not done.

4.5. Determination of Fibre Type Distribution

All slides were viewed with a Zeiss photomicroscope. Pictures were taken of all suitable sections. The percentage of type I and type II fibres was determined from the photographs. The number of fibres counted was 150 to 300; samples with less than 150 fibres were discarded.

4.6. Determination of Fibre Size

Individual fibre area was determined using a Tektronix 4006-1 Cybergraph System (Talos Systems Inc.). Analysis was done from the projected image of each slide. Ten fibres of each type were analyzed per subject (Thorstensson 1976). All sections counted were those incubated at pH 9.4. Only fibres that were cut in true cross-section were selected.
4.7. Statistical Analysis

Two statistical tests were performed to determine the significance of the data; a standard t-test and a linear regression plot.
RESULTS
RESULTS

5.1. Comparisons of Fibre Type and Performance

A regression line plot and correlation was used to compare the results of each of the three performance tests with each of the four muscle fibre analyses. These comparisons are presented in Tables 1 to 12 and Figures 1 to 12. The required r value for all correlations was .567 at the 5% confidence level.

Isometric strength was compared to type I fibre size (Table 1, Fig. 1). The r value was not significant (-.232). Table 2 and Fig. 2 present the comparison between isometric strength and type II fibre size. The r value was found to be .232, indicating that there was no significant relationship between these two variables. When isometric strength was plotted against percentage of type II fibres the observed correlation coefficient was .247 (see Fig. 3 and Table 3). This value was not significant. Isometric strength was also compared to the percentage of type II fibre area (Table 4, Fig. 4). The observed r value (.365) was not significant at the 5% confidence level.

The individual scores for endurance repetitions were also compared to the four muscle fibre measurements. When type I fibre size was considered there was no significant correlation with endurance repetitions. The r value obtained was -.231 (see Table 5 and Fig. 5). The comparison between endurance repetitions and type II fibre size is presented in Table 6 and Fig. 6. The observed r
### Table 1: Regression of isometric strength on type I fibre size.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required $r$</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>-0.232 (.054)</td>
<td>0.567 @ 5%</td>
<td>significant</td>
</tr>
<tr>
<td>$n = 12$</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Regression of isometric strength on type II fibre size.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required $r$</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>0.232 (.054)</td>
<td>0.567 @ 5%</td>
<td>significant</td>
</tr>
<tr>
<td>$n = 12$</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Regression of isometric strength (y) on type I fibre size (x).

Figure 2: Regression of isometric strength (y) on type II fibre size (x).
Field hockey

Volleyball

\[ y = -0.003x + 75.0 \]

\[ y = 0.002x + 53.9 \]
Table 3: Regression of isometric strength on percentage of type II fibres.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required $r$</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
<td></td>
<td>not</td>
</tr>
<tr>
<td>$n = 12$</td>
<td>10</td>
<td>.247 (.061)</td>
<td>.567 @ 5%</td>
<td>significant</td>
</tr>
</tbody>
</table>

Table 4: Regression of isometric strength on percentage type II fibre area.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required $r$</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
<td></td>
<td>not</td>
</tr>
<tr>
<td>$n = 12$</td>
<td>10</td>
<td>.365 (.133)</td>
<td>.567 @ 5%</td>
<td>significant</td>
</tr>
</tbody>
</table>
Figure 3: Regression of isometric strength \( (y) \) on percentage of type II fibres \((x)\).

Figure 4: Regression of isometric strength \( (y) \) on percentage of type II fibre area \((x)\).
Table 5: Regression of endurance repetitions on type I fibre size.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required $r$</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>-.231 (.053)</td>
<td>.567 @ 5%</td>
<td>significant</td>
</tr>
<tr>
<td>n = 12</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Regression of endurance repetitions on type II fibre size.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required $r$</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>.155 (.024)</td>
<td>.567 @ 5%</td>
<td>significant</td>
</tr>
<tr>
<td>n = 12</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5: Regression of endurance repetitions \((y)\) on type I fibre size \((x)\).

Figure 6: Regression of endurance repetitions \((y)\) on type II fibre size \((x)\).
The diagram shows two sets of data points for.Field hockey and Volleyball, plotted against the Type I and Type II fibre size in micrometers squared. 

For Type I fibre size (μm²):
- The linear equation is \( y = -0.02x + 152.49 \).
- The data points are scattered along the line indicating a negative correlation between fibre size and endurance repetitions.

For Type II fibre size (μm²):
- The linear equation is \( y = 0.01x + 45.88 \).
- The data points are scattered along the line indicating a positive correlation between fibre size and endurance repetitions.

The graph visually represents the relationship between fibre size and endurance repetitions for the two types of sport.
value of .155 was well below the required $r$ value of .567 for significance at the 5% confidence level. There was no significant relationship between endurance repetitions and the percentage of type II fibres, or between endurance repetitions and the percentage of type II fibre area (see Tables 7 and 8, Figs. 7 and 8). The observed $r$ values for these two comparisons were .159 and .255 respectively.

Table 9 and Fig. 9 present the comparison between vertical jump and type I fibre size. The observed $r$ value was -.482. This was not significant at the 5% confidence level. A comparison of vertical jump and type II fibre size revealed no significant correlation between these two variables ($r = -.076$) (see Table 10, Fig. 10). When vertical jump was plotted against the percentage of type II fibres (Fig. 11) an $r$ value of .312 was obtained (Table 11). This was not significant. No significant correlation was found when vertical jump was compared to the percentage of type II fibre area (Fig. 12). As is shown in Table 12, the observed $r$ value for this comparison was .376.

To summarize the data presented in Tables 1 to 12 and Figs. 1 to 12; there was no significant correlation between individual comparisons of the three performance tests and the four measurements of muscle fibre size or percent distributions.

Regression line correlations were also used to investigate any interrelationship among the three performance tests. No significant correlations at the 5% confidence level were found between
isometric strength and vertical jump, isometric strength and endurance repetitions, or vertical jump and endurance repetitions (see Table 13).
Table 7: Regression of endurance repetitions on percentage of type II fibres.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>.159 (.025)</td>
<td>.567 @ 5% significant</td>
</tr>
<tr>
<td>n = 12</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8: Regression of endurance repetitions on percentage type II fibre area.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>.255 (.065)</td>
<td>.567 @ 5% significant</td>
</tr>
<tr>
<td>n = 12</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 7: Regression of endurance repetitions \((y)\) on percentage of type II fibres \((x)\).

Figure 8: Regression of endurance repetitions \((y)\) on percentage of type II fibre area \((x)\).
O Field hockey  ▲ Volleyball

\[ y = 0.74x + 42.04 \]

\[ y = 1.03x + 26.99 \]
### Table 9: Regression of vertical jump on type I fibre size.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ( (r^2) )</th>
<th>Required ( r )</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>-0.482 (.232)</td>
<td>0.567 @ 5%</td>
<td>significant</td>
</tr>
<tr>
<td>( n = 12 )</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 10: Regression of vertical jump on type II fibre size.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ( (r^2) )</th>
<th>Required ( r )</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>-0.076 (.006)</td>
<td>0.567 @ 5%</td>
<td>significant</td>
</tr>
<tr>
<td>( n = 12 )</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 9: Regression of vertical jump \( (y) \) on type I fibre size \( (x) \).

Figure 10: Regression of vertical jump \( (y) \) on type II fibre size \( (x) \).
O Field hockey  ▲ Volleyball

\[ y = -0.003x + 49.15 \]

\[ y = -0.00x + 38.21 \]
Table 11: Regression of vertical jump on percentage of type II fibres.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required $r$</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>.312 (.097)</td>
<td>.567 @ 5%</td>
<td>significant</td>
</tr>
<tr>
<td>$n = 12$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 12: Regression of vertical jump on percentage type II fibre area.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Observed Correlation Coefficient ($r^2$)</th>
<th>Required $r$</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td>.376 (.141)</td>
<td>.567 @ 5%</td>
<td>significant</td>
</tr>
<tr>
<td>$n = 12$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 11: Regression of vertical jump (y) on percentage of type II fibres (x).

Figure 12: Regression of vertical jump (y) on percentage of type II fibre area (x).
Table 13: Regression line comparisons between the three performance measurements.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Observed $r$ ($r^2$)</th>
<th>Required $r$ @ 5%</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isometric strength vs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical jump</td>
<td>-.015 (.000)</td>
<td>.567</td>
<td>not significant</td>
</tr>
<tr>
<td>Isometric strength</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs. Endurance repetitions</td>
<td>.381 (.145)</td>
<td>.567</td>
<td>not significant</td>
</tr>
<tr>
<td>Vertical jump</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs. Endurance repetitions</td>
<td>.360 (.130)</td>
<td>.567</td>
<td>not significant</td>
</tr>
</tbody>
</table>
5.2. Subgroup Comparisons

A standard t-test was used to compare the height, weight, weight/height ratios, and ages of the volleyball and field hockey groups (see Table 14). There were significant differences in age (p < 0.05) and height (p < 0.01) between the two groups. No significant difference was found with respect to weight or weight/height ratio.

Table 15 presents the performance measurements for the two groups. There was no significant difference between the volleyball players and the field hockey players with respect to endurance repetitions or isometric strength. There was, however, a significant difference found in the vertical jump scores (p < 0.05).

For each of the twelve subjects the percentage of type I and type II fibres was calculated. As presented in Table 16, there was no significant difference between the two groups with respect to percentage distribution of type II fibres.

Mean fibre size of type I and type II fibres for both groups is presented in Table 17. The mean size of the type I fibres in the field hockey group was significantly larger than the mean type I fibre size in the volleyball group (p < 0.001). There was no significant difference between the two groups with respect to type II fibre size.

When the percentage of area occupied by type II fibres was calculated it was found that the volleyball group had a significantly greater percentage of type II fibre area (p < 0.05).
Table 14: Age, Weight, Height, and Weight/Height ratio measurements* of volleyball and field hockey players.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age</th>
<th>Weight (Kg.)</th>
<th>Height (cm.)</th>
<th>Weight/Height Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volleyball</td>
<td>4</td>
<td>18.5 ± .29a</td>
<td>63.7 ± 3.2</td>
<td>174.5 ± 2.6c</td>
<td>.366 ± .02</td>
</tr>
<tr>
<td>Field Hockey</td>
<td>8</td>
<td>20.5 ± .73b</td>
<td>59.1 ± 2.4</td>
<td>159.8 ± 1.97d</td>
<td>.381 ± .02</td>
</tr>
</tbody>
</table>

* Values are mean ± S.E.M.

b > a (p < 0.05)
c > d (p < 0.01)
Table 15: Performance measurements* for isometric strength, vertical jump, and endurance repetitions.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Isometric Strength (Kg.)</th>
<th>Vertical Jump (cm.)</th>
<th>Endurance Repetitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volleyball</td>
<td>4</td>
<td>68.6 ± 4.5</td>
<td>41.3 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117.8 ± 43.7</td>
</tr>
<tr>
<td>Field Hockey</td>
<td>8</td>
<td>61.5 ± 3.3</td>
<td>33.96 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.9 ± 12.3</td>
</tr>
</tbody>
</table>

* Values are mean ± S.E.M.

a > b (p < 0.05)
Table 16: Percentages* of type II fibres and type II fibre area.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>% Type II fibres</th>
<th>% Type II fibre area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volleyball</td>
<td>4</td>
<td>64.0 ± 3.8</td>
<td>66.8 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Field Hockey</td>
<td>8</td>
<td>52.3 ± 4.6</td>
<td>49.7 ± 5.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values are mean ± S.E.M.

<sup>a</sup> > <sup>b</sup> (p < 0.05)
Table 17: Type I and type II fibre sizes*.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Type II fibre size (μm.²)</th>
<th>Type I fibre size (μm.²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volleyball</td>
<td>4</td>
<td>4119.5 ± 145.3</td>
<td>3636.9 ± 107.0⁴</td>
</tr>
<tr>
<td>Field Hockey</td>
<td>8</td>
<td>3956.7 ± 101.4</td>
<td>4362.7 ± 101.1⁵</td>
</tr>
</tbody>
</table>

* Values are mean ± S.E.M.

b > a  (p < 0.001)
DISCUSSION
DISCUSSION

Numerous studies have been conducted regarding muscle fibre composition in trained and untrained male subjects. However, very little information exists with respect to females. The few studies that have been done indicated that females, in general, have smaller muscle fibres, although muscle composition and fibre distribution are similar in both sexes (Costill et al. 1976a, Prince et al. 1977, Taylor et al. 1978). In an effort to gain additional data it was decided to choose only female subjects for the present study.

Performance tests were selected to evaluate three basic parameters: muscular strength, muscular endurance, and power. Because the biopsies were taken from the right vastus lateralis, an effort was made to isolate the quadriceps femoris muscles in these tests to facilitate the comparison between fibre types and performance. Therefore, the isometric strength and endurance tests were performed on the right lower limb. The vertical jump test for power was selected because of its proven reliability, and the fact that data on the relationship between vertical jump and fibre type composition are sparse.

Regression line comparisons among the performance tests showed that a significant correlation did not exist between isometric strength and vertical jump, isometric strength and endurance repetitions, or vertical jump and endurance repetitions. Because the repetitions are a measure of anaerobic endurance, it is not surprising that there was no correlation between them and the scores on isometric strength or
vertical jump. However, power and strength empirically appear to be more interdependent because strength is a component of power; power being the rate with which work is performed (devries 1974). There are other factors that must be considered, such as speed of movement and body weight of the individual. When weight/height ratios were plotted against vertical jump scores a negative regression line correlation was obtained (p < 0.05). Athletes who were proportionately lighter for their height scored better on the vertical jump test. One explanation for this may be that these subjects have to overcome proportionately less inertia.

Regression line correlations were also plotted to individually compare the three performance tests with the four components of muscle fibre analysis: percentage of type II fibres, percentage of type II fibre area, size of type I fibres, and size of type II fibres.

When endurance repetitions were plotted against each of the fibre analyses no significant correlation was found with any of the four. Although this appears to be contradictory to previous studies that indicated increased type I fibre percentage or area percentage in endurance trained athletes, few studies have been published that actually compare fibre type distribution to objective measures of endurance. The present observations do concur with those of Burke et al. (1977) who did not find a correlation between V0₂max. and percent ST fibres in male and female competitive cyclists.

On the other hand, Bergh et al. (1978) noted a positive corre-
lation between VO₂ max. and percentage of type I fibres in elite athletes and moderately trained subjects. The correlation coefficient for the elite athletes was .72 (p < 0.01), but for the moderately trained group the r value was only .34 (p < 0.05).

Costill et al. (1973) claimed to have found a correlation between percentage of type I fibres and VO₂ max., although a statistical analysis was not indicated. However, in a subsequent study, Costill et al. (1976a) observed that the percentage of type I fibres showed little relationship to VO₂ max.

The controversy and conflicting evidence on the relationship between fibre type distribution and endurance capacity, as measured by VO₂ max., is obvious. What these studies may indicate is that fibre composition is a factor in endurance performance only in very highly trained athletes. In moderately trained athletes, many other factors must be taken into account.

Peripheral adaptations such as capillarization and oxidative enzymes within the muscle are important for oxygen exchange and utilization. With endurance training, increased capillarization, and then increased SDH activity, are the sequence of events (Brown et al. 1976). These factors may limit endurance, and yet are not accounted for by simply classifying fibres as type I or II with the myosin ATPase technique.

In addition to adaptations within the muscle, the functioning efficiency of the heart and lungs must be considered. However,
discrepancies between VO₂ max. and muscle fibre composition may occur because capillarization and SDH changes precede central changes. Therefore, the failure to correlate VO₂ max. with fibre composition may, in part, be a reflection of this time lag between central and peripheral adaptations.

The Sargent jump test was used to measure power. When a regression line plot was used to compare the power test results with the muscle fibre parameters, no significant correlation was found with any of the four. This is in agreement with the findings of Campbell et al. who did not observe any relationship between vertical jump and percentage of type II fibres. This group also found no correlation between the percentage of type II fibres and three other measures of power: a heavy power test, a light power test, and the Lewis Power Index. In a study of monozygous and dizygous twins, Komi and Karlsson (1979) also failed to observe any relationship between percent type I fibres and power, as measured by the anaerobic power test of Margaria et al. (1966). They did note, however, a high positive correlation between power and percent body fat. In a more recent investigation of male physical education students, Bosco and Komi (1979) noted a positive correlation between the percentage of type II fibres and height of rise of centre of gravity from a squatting jump.

These studies provided the only objective information with respect to the relationship between fibre type and power. Although
little published evidence exists of a significant dependence of power on fibre type distribution, some researchers maintain that vertical jump is an indicator of type II fibre content, and further, that it is related to the trainability of athletes (Councilman 1979). There is, to date, no evidence that fibre type distribution can be predicted merely from vertical jump scores, or from any other objective performance variables alone or in combination.

The third performance parameter measured was isometric strength. When it was plotted individually against percent of type II fibres, percent of type II fibre area, and type I and II fibre sizes, using a linear regression comparison, any significant correlations were not apparent.

The present observations add to the controversy that prevails in the literature regarding strength and muscle fibre type. Komi and Karlsson (1979) noted a significant negative correlation between isometric strength and percent of type I fibres. Tesch and Karlsson (1978) observed a positive linear correlation between the percent of type II fibres or percent of type II fibre area and isometric strength in thirty-one physical education students. There are, however, a substantially greater number of studies where no correlation between isometric strength and fibre type distribution was found (Hulten et al. 1975, Thorstensson 1976, Thorstensson et al. 1976, Dons et al. 1979).

It has already been established that, in human subjects, both FT and ST motor units are involved in the production of static,
isometric tension (Gydikov and Kosarov 1974). In combination with the above studies, where a correlation between isometric strength and fibre type was not observed, this may indicate that type I and type II fibres are involved to an equal extent in isometric force production (Thorstensson 1976).

There is, however, evidence indicating that dynamic strength is dependent on type II fibre content. Studies employing isokinetic devices demonstrated a positive correlation between the percentage of type II fibres and the speed of joint movement. This correlation becomes increasingly significant as the speed of movement increases (Thorstensson et al. 1976, Coyle et al. 1979).

Subgroup Comparisons

The purpose of this investigation was to relate fibre type distribution in trained females to specific measurements of physical performance. For the main study all subjects were treated as one group. Each of the volunteers was a member of either an intercollegiate volleyball team or intercollegiate field hockey team. This allowed for comparison of performance scores and fibre type distributions between these two subgroups.

The two subgroups were selected on the basis of the different training demands for each sport. Field hockey, being a running game, requires endurance (continuous, submaximal workloads). In contrast, volleyball is a jumping game, workloads are maximal but of short duration. Because of this it was felt that the two groups were
representative of activities that stressed the two different metabolic systems (aerobic and anaerobic) as well as the two basic muscle fibre groups.

The data on the height, weight, and ages of the two groups indicated that the volleyball players were significantly taller and younger than the members of the field hockey group. The difference in height can undoubtedly be explained by the selection process for the two teams. It is advantageous for volleyball players to be tall because of the physical obstacle the height of the net presents. This is not true of field hockey, and hence it is not surprising that the mean height of the field hockey players more closely paralleled that of the normal population. The difference in age between the two groups, although statistically significant, probably has no bearing on either the performance test results or the muscle fibre analysis. Studies on the differentiation of fibre types and the aging process revealed that there was no difference in fibre type distribution between a two year old child and an adult, and that age did not become a factor until the fourth or fifth decade when the type II fibre area begins to decrease (Curless 1977, Larsson and Karlsson 1978).

Fibre type distributions were determined in muscle biopsies from twelve subjects. The original group was composed of six volleyball players and nine field hockey players. Percentages of type I and type II fibres were determined by examining a minimum of 150 fibres per subject and, with the exception of two subjects, all histologic sections contained at least 200 fibres (Thorstensson 1976, Tesch et
al. 1978). Because of this restriction three subjects were discarded from the group due to small biopsy specimens, reducing the total number of subjects to twelve: four volleyball players and eight field hockey players.

There was no significant difference between the mean percentage of type II fibres in the volleyball group and mean percentage of type II fibres in the field hockey group (0.1 > p > 0.05). However, when the percentage of area occupied by type II fibres was considered, a significant difference between the two groups was found (p < 0.05). The factor affecting the percentage area determination between the two groups was the individual size of the fibre types.

A standard t-test, comparing the size of the volleyball group's type II fibres with the field hockey group's type II fibres, revealed no significant difference. There was, however, a highly significant difference between the sizes of the type I fibres. The field hockey group had significantly larger type I fibres (p < 0.001). This may be interpreted in one of two ways.

There may be a selective hypertrophy of type I fibres in the field hockey group, or the difference may simply be due to natural selection. Because of the emphasis upon cardiovascular and muscular endurance in the field hockey training schedule, it is likely that the type I fibres are selectively recruited because of their predominantly aerobic metabolism (Andersen and Sjogaard 1975, Green 1978). Hypertrophy due to selective motor unit recruitment is supported by Gollnick et al.
(1973) who showed an increase in type I fibre size and percentage area with training. Moreover, it has been indicated that selective fibre hypertrophy occurs in strength training. Costill et al. (1979) demonstrated that seven weeks of isokinetic training resulted in a significant increase in the percentage of type II fibre area in five male subjects.

The other possibility is that the difference in fibre size and percentage area is genetic. It may be that the athletes with larger type I fibres are more successful in endurance activities and are therefore chosen for the field hockey team. The difference, in this case, would be one of natural selection as opposed to adaptation. Investigations into the effects of training on fibre type size and distribution, as classified by the myosin ATPase technique, have not all revealed this phenomenon of selective hypertrophy. In two separate studies, Thorstensson did not observe any change in the percentage distribution of the two fibre types after sprint training (1975) or strength training (1976). He did find an increase in the ratios of type II to type I fibre areas in the strength trained group. There was no significant increase in the mean size of both fibre types after sprint training, although he did note a trend in that direction. However, since there were only three subjects in the group a more definitive conclusion regarding statistical significance cannot be made until greater numbers of individuals are tested.

When the results of all the correlations are considered, they cast
doubt upon theories that are in general acceptance by many researchers. Perhaps muscle fibre type is not as significant a factor in athletic performance as it is often held to be. Simple fibre typing is in no way indicative of the functional efficiency of the nervous system. Smoothness and efficiency of motor unit recruitment, influence of upper motor neurons, and sensory input will all affect performance of a skilled task. Genetic factors, such as skeletal frame, insertion of muscles, angle of pull on bones, length of body parts may help to determine athletic success. Other elements that are difficult, or impossible, to evaluate (such as motivation, anticipation, concentration, etc.) may make an equally important contribution to skilled athletic performance.

It is clear that the significance of the various fibre types must be investigated through specific measures of performance. Extrapolation from case studies of elite athletes culminates in speculation but does not yield definitive answers. Consequently, more research is required to firmly establish the role of fibre types, not only in athletic performance, but in the general population as well.
CONCLUSIONS
CONCLUSIONS

Within the limitations of this study, the following conclusions seem justified:

1. Isometric strength is not related to fibre type distribution.
2. Power is not related to fibre type distribution.
3. Muscular endurance is not related to fibre type distribution.

Comparisons between field hockey and volleyball players revealed that:

1. Volleyball players had a significantly greater percentage area of type II fibres.
2. Field hockey players had significantly larger type I fibres.
3. Differences in fibre type could not be related to performance.
BIBLIOGRAPHY


