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

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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.

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

REVIEW OF LITERATURE

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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 chielfy 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 analagous 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 $\alpha\beta$ 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.

	Reactivity for Myosin ATPase (pH 9.4)	Reactivity for Oxidative Enzymes	Reactivity for Glycolytic Enzymes
ST	low	high	low
Type I	low	high	low
FT	high	low to high	moderate to high
Type II	high	low to high	moderate to high
SO	low	high	low
Type I	low	high	low
FOG	high	moderate to high	moderate
Type IIA	high	moderate to high	moderate
FG	high	low	high
Type IIB	high	low	high

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 Kaijser 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 μm.^{2}, while type II fibres range from 3500 to 7500 μm.^{2}. In trained male athletes, type I fibres may range from 5000 to 9000 μm.^{2}, while type II fibres range from 5000 to 10,000 μm.^{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 $\mu m.^2$, (Prince et al. 1977) and 3875 $\mu m.^2$ (Costill et al. 1976a), while values for FG and FOG fibres were 2425 $\mu m.^2$ and 3392 $\mu m.^2$ (Prince et al. 1977), and FT fibres were 4193 $\mu m.^2$ (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 $\mu m.^2$ (Costill et al. 1976a, Burke et al. 1977, Prince et al. 1977). However, average values as large as 9003 $\mu m.^2$ for ST fibres and 8557 $\mu m.^2$ 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 Sjogaard 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_2max. (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 add-
ition, there was no indication of an increase in FT area/ST area
even though the increase in VO_2max. (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 bio-
chemical 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