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THE HISTOCHEMICAL DIFFERENTIATION OF FIBRE TYPES IN ENDURANCE TRAINED AND SEDENTARY INDIVIDUALS USING A FORMATE KCl BUFFER

A Thesis Presented to the

Faculty of University Schools

Lakehead University

In partial fulfillment of the requirements for the

Degree of Master of Science in

Applied Sport Science and Coaching

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ABSTRACT

Title of Thesis: The Histochemical Differentiation of Fiber Types in Endurance Trained and Sedentary Individuals Using a Formate-KCl Buffer

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Endurance training produces a fast-to-slow fibre transformation as well as an increase in the number of transitional fibres. To further examine these findings, skeletal muscle from seven decade-trained aerobic (DT) and six non-trained (NT) subjects was obtained by a muscle biopsy from the vastus lateralis (VL). A preliminary histochemical study completed by Thayer and Fanti (1992) exposed muscle tissue to an alkaline preincubation (pH 9.9) (Padykula & Herman, 1955), an acid preincubation (pH 4.3 or 4.6)(Brooke and Kaiser, 1970b) and a formate-KCl preincubation buffer (pH 4.54) (Matoba & Gollnick, 1984) to determine the reliability of the formate KCl buffer to identify all fibre types in human skeletal muscle. In the present study the muscle tissue was histochemically treated by exposure to both an alkaline preincubation (pH 9.9) (Padykula & Herman, 1955; Brooke & Kaiser, 1970b) and the formate-KCl preincubation buffer (pH 4.54), previously employed by Matoba & Gollnick (1984). The percentage of histochemically identified

type I fibres in DT was 70.88% vs 37.73% in NT ($p<0.01$), while the type IIa in DT (25.25%) was much lower ($p<0.01$) than the NT (51.84%) and transitional fibre type IIbaL (acid labile) in the DT (3.46%) was lower ($p<0.05$) than in the NT (8.53%). Surprisingly, type IIa fibres in the NT group displayed greater oxidative staining intensity ($p<0.01$) than type IIa fibres from the DT group. The results revealed that endurance training may promote a transition in fibre types from fast-to-slow and may occur at the expense of the type IIa and type IIb fibre population. In addition, the application of the formate KCl buffer to human skeletal muscle provided a reliable method for identification of the major fibre types.

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CHAPTER 1

INTRODUCTION

Research Hypothesis

The number of transitional/intermediate fibre types is more predominant in decade trained endurance athletes as compared to sedentary individuals and the oxidative capacity at the fibre level in the trained muscle is greater than that of the non-trained muscle.

The Purpose of the Study

The purposes of this paper are to (1) determine the efficacy of a formate KCl buffer in identifying the fibre types in human skeletal muscle (2) determine if the number of transitional fibres are more predominant in decade trained endurance athletes compared to sedentary individuals, and (3) compare the oxidative and glycolytic potential of muscle fibres obtained from these subjects.

Significance of the Study

Previous research has confirmed that mammalian skeletal muscle could be divided into type I, type IIa and type IIb fibres using the myofibrillar actomyosin adenosine triphosphatase (ATPase) staining procedure as originally proposed by Brooke and Kaiser (1970). The type I fibres accommodate slow-contracting motor units, type IIa fibres accommodate fast-contracting fatigue-

resistant motor units, and type IIb fibres accommodate fast-contracting fatigue-sensitive motor units (Burke, Levine, Tsairis & Zajac, 1973; Matoba, Allen, Bayly, Oakley, & Gollnick, 1985; Pernus & Erzen, 1991). Modifications in histochemical procedures (Gollnick & Matoba, 1984a; Matoba et al., 1985) advancement in the biochemical (Staron & Pette, 1987a; Staron & Pette, 1987b; Termin, Staron & Pette, 1989) and immunohistochemical (Kucera, Walro, & Gorza, 1992; Schiaffino, Gorza, Sartore, Saggin, Ausoni, Vianello, Gundersen & Lomo, 1989) techniques have identified a continuum of staining intensities between type I and type IIb fibres.

Researchers have confirmed the plasticity of skeletal muscle by utilizing techniques such as cross-reinnervation (Close, 1969; Barany & Close, 1971), chronic electrical stimulation (Gorza, Gundersen, Lomo, Schiaffino, & Westgaard, 1988) and compensatory overload (Ianuzzo, Gollnick & Armstrong, 1976; Roy, Baldwin, Martin, Chimarusti & Edgerton, 1985). The excess stress from these techniques demonstrated that fibres are quite dynamic within a given muscle and could transform from one type to another depending on the extent of the stress applied.

Human studies have demonstrated that with endurance training there would be a fast-to-slow fibre type transformation, ultimately resulting in the predominant expression of slow myosin light and heavy chains (Alway, MacDougall, Sale, Sutton, & McComas, 1988; Rusko, Bosco, Komulainen, Leinonen, & Vihko, 1991; Green, Jones,

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Ball, Smith, Livesey, & Farrance, 1991; Maier, Gorza, Schiaffino, & Pette, 1988; Baumann, Jaggi, Soland, Howald, & Schaub, 1987). Moreover, in addition to the typical transformation in human fibres between type I and type II or intermediate fibre types several investigators have identified the presence of transitional fibres in human skeletal muscle. Baumann, Jaggi, Soland, Howard and Schaub (1987) detected some training-induced changes in the peptide pattern of the myosin heavy chains in the type IIa fibres. Based on the myosin heavy chain isoforms, some transitional tissue was identified as the type IIa and type IIc fibres which marked the beginning of their transition towards the slow type I fibre. Furthermore, Baumann et al. (1987) observed some variation in staining intensity in other fibre types which may represent minor quantitative differences in the peptide pattern.

Using strength-trained women, Staron and Hikida (1992), using myosin heavy and light chains and mitochondrial volume, identified some transitional fibres which appeared to represent a continuum of type C fibres. The type C fibres were histochemically subdivided into three main fibre types: IC, IIC, and IIAC. The IC fibres were histochemically more similar to the typical type I fibre, and the IIAC were more similar to the type IIa fibre. As well, the C fibre population revealed an oxidative capacity between fibre types I and type IIa and confirmed the existence of transitional or intermediate fibres.

Preliminary work by Thayer and Fanti (1992) compared the

staining pattern for myofibrillar actomyosin ATPase with a formate-KCl buffer (pH 4.6). They were able to distinguish a subset IIbL of the type IIb fibre population. It was concluded that this method was reliable when compared to the standard histochemical methods and was preferred because the subclassification of fibre types could be obtained at a single pH (Matoba & Gollnick, 1984; Thayer & Fanti, 1992).

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Limitations

1. Muscle tissue was incubated for various times in a formate-KCl buffer (pH 4.6).
2. Samples of tissue were placed as rapidly as possible into 2-methyl butane and immediately pre-cooled by liquid nitrogen.
3. Samples were stored at -80°C until analyzed.
4. The exact training background of the athletes was not monitored.
5. The activity background of the sedentary group was quite varied.
6. No attempt was made to control diet
7. Only one site in the vastus lateralis was used to obtain the three muscle samples.

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Delimitations

1. Only males were used in the study.
2. Subjects in the decade trained group were not randomly selected. Only well trained athletes who have been training aerobically for a period of a decade or more were employed during the study.
3. Alpha-glycerophosphate dehydrogenase and nicotinamide adenine dinucleotide tetrazolium reductase were used to demonstrate glycolytic and oxidative capacities respectively.
4. Staining intensity for myofibrillar actomyosin ATPase was observed at an alkaline preincubation at pH 9.9 (Padykula & Herman, 1955; Brooke & Kaiser, 1970b).
5. A Formate-KCl buffer was used as the preincubation medium to sequentially inactivate the myofibrillar actomyosin ATPase at time intervals of 10s, 40s, 90s, 150s, and 240s.
6. Statistical significance level of .05 was used.
7. Dependent measures are fibre types, oxidative and glycolytic capacities.
8. Fibres were categorized by staining intensity using a 1-4 scale.
9. The muscle biopsy was taken from the vastus lateralis.

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Definitions

Alpha-glycerophosphate dehydrogenase - A glycolytic marker enzyme which assists in the process of glycolysis.

Compensatory hypertrophy model - Surgical removal or tenotomy of a synergistic muscle to observe any transformation of the remaining muscle.

Cross reinnervation - Taking the motor nerve from a typical slow or fast muscle and reuniting it to a muscle with opposing contractile characteristics.

Electrical stimulation: Positioning of two electrodes within a muscle and stimulating it with similar motor nerve impulses carried by either slow or fast muscle.

Fascicle - a bundle of muscle fibres enclosed by a connective tissue sheath called the perimysium.

Monoclonal antibody - Homogeneous immunoglobulin derived from a single clone of cells.

Myofibrillar adenosinetriphosphate (ATPase) - This enzyme splits ATP to produce energy used for muscle contraction.

Nicotinamide-adenine dinucleotide (NAD^+) - Coenzyme that accepts two electrons and one proton to generate the reduced form, NADH; important electron carrier in energy metabolism.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis - Technique used to separate proteins based on two parameters, electrical charge and size.

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Transitional fibres - Type IIbaS - more acid stable

Type IIbaL - more acid labile

Type IIbaS (more acid stable) - is a fibre type which stains using conventional myosin ATPase histochemistry as a type II fibre at preincubations of pH of 10.3 and 4.3, and as a type IIb fibre at a preincubation pH of 4.6.

Type IIbaL (more acid labile) - is a fibre type which stains using conventional myosin ATPase histochemistry as a type II fibre at a preincubation pH of 4.6. But at pH of 4.6 the type IIbaL fibre stains with less intensity than the type IIbaS fibre.

CHAPTER 11

LITERATURE REVIEW

A great deal of attention over the past several decades has been focused on the study of mammalian muscle tissue through histochemical analysis. The oldest classification of muscle tissue was based on gross appearance. Muscles such as the soleus appeared red while others such as the lateral head of the gastrocnemius, appeared white. Muscle fibres which were most numerous in red muscle were called red fibres while those most numerous in white muscle were called white fibres (Brooke & Kaiser, 1970b).

Major advancements in the application of histochemistry occurred in the field of muscle pathology with the development of a glycolytic stain (Wattenberg & Leong, 1960) which identified alpha-glycerophosphate dehydrogenase (α-GPDH) activity and shortly thereafter the development of an oxidative stain, nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) (Novikoff, Schin, & Drucker, 1961). With the development of these stains it was possible to differentiate fibre types based on oxidative and glycolytic potential.

At approximately the same time, Dubowitz (1960) classified muscle fibres as type I or type II based on the change in the intensity of the ATPase staining in relation to time, temperature, and pH preincubations. It was observed that when the muscle fibre was preincubated outside of a given range of pH, the histochemical

reaction for ATPase would fail to develop in that fibre (Rosen, 1970). The ATPase reaction of type I fibres was inhibited if preincubation was outside the range of pH 3.9 to 10.4, type IIa was pH 4.9 to 10.8, and type IIb was pH 4.5 to 10.8. In addition, a small group of type IIc fibres were inhibited at pH 4.9 to 10.8 (Brooke & Kaiser, 1970b; Rosen, 1970). Researchers have demonstrated that the marked differences in staining characteristics of fibres using the myosin ATPase reaction are dependent upon very small differences in pH lability of the enzyme system (Bass, Brdiczka, Eyer, Hofer, & Pette, 1969: Gollnick, Parsons & Oakley, 1983).

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Table 1:Histochemical and biochemical properties of different fibre types

Property	Fibre type				
	I	IIa	IIc	IIb	Reference
Enzyme activity					
Myofibrillar ATPase	low	high	high	high	1
Oxidative enzymes	high	intermediate-high	intermediate	low	2
Glycolytic enzymes	low	high	high	high	3
Substrate concentration					
ATP	intermediate	high	intermediate	high	4
CP	intermediate	high	high	high	4
Glycogen ■	low	intermediate	high	intermediate	2
Triglycerides	high	-	-	low	2

■ In human skeletal muscle, glycogen concentrations are similar in all fibre types.

[1]Ingjer, 1979; [2]Brooke and Kaiser, 1970a; [3]Kreighbaum and Barthels, 1985; [4]Bass, Brdiczka, Eyer, Hofer, Pette, 1969.

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As outlined in Table 1, type I and type II fibres have many different histochemical and biochemical characteristics which are used when discussing a particular fibre type. Type I fibres are high in oxidative potential, type IIa fibres are intermediate, and type IIb fibres are low. The reverse is true for glycolytic potential; type I fibres are low in glycolytic potential and type IIa and type IIb are high. However, with the advancement in the study of skeletal muscle, new histochemical, biochemical and immunocytochemical techniques have been developed to monitor changes in response to stress.

Histochemical methods used for identifying and studying the muscle fibre types have increased in sophistication over the past decade to provide as much information as possible about the similarities and differences between fibre types. For example, Gollnick, Parsons, and Oakley (1983), developed a method for identifying fibre types of skeletal muscle on the basis of the sequential inactivation of myofibrillar actomyosin ATPase during acid preincubation. When this method was used some form of type I and type II transitional fibres, with slight staining variations from the traditional fibre types, were identified. In addition, Gollnick and Matoba (1984a), developed a preincubation stain which comprised of low concentrations of copper (Cu^{2+}) at neutral (7.40), acid (4.60), and alkaline (10.3) pH. The researchers demonstrated that the inclusion of Cu^{2+} in the preincubation media for the myofibrillar actomyosin ATPase can also be a useful tool to

differentiate fibre types.

Gollnick and Matoba (1984b) completed a study in muscle histochemistry by manipulating the preincubation medium with the addition of buffering compounds and neutral salts and by altering the medium's pH levels. When the carboxylic acid had multiple carboxyl groups, the myofibrillar actomyosin ATPase reaction was accelerated. With the addition of a citrate buffer, a sharp differentiation between fibre types was produced, whereas in contrast, the addition of an acetate buffer produced similar histochemical results but increased the time course for differentiation of the fibre types. However, with the addition of neutral salts to the preincubation medium, the activation time was accelerated. The researchers concluded that the histochemical differentiation of fibre types can be influenced by a number of factors.

Preliminary research conducted by Thayer and Fanti (1992), using human skeletal muscle, compared the staining pattern for myofibrillar actomyosin ATPase with a formate-KCl buffer (pH 4.6) as the preincubation medium. They found not only did it clearly differentiate the same fibre types as the alkaline and acid preincubations, but it did so in one preincubation step. The formate-KCl buffer also made it possible to clearly distinguish in human skeletal muscle a subset of the type II fibre population, which was designated as a IIbL fibre. This fibre type stained as

a type IIb with the standard preincubation at a pH of 4.6, but displayed an oxidative capacity similar to a type IIa fibre.

A biochemical technique which has been used extensively over the past decade, in conjunction with conventional histochemical techniques, to more accurately differentiate fibre types is sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (Staron & Pette, 1987a; Staron & Pette, 1987b; Termin, Staron & Pette, 1989). By employing this procedure fibre types can be more clearly differentiated on the basis of their contractile protein composition.

Staron and Pette (1986) used histochemical and electrophoretic techniques on single fibres of rabbit soleus muscle to identify four fibre types (I, IC, IIC, and IIA). Histochemically types I and IIA were separate homogeneous groups, while a heterogeneous C fibre population exhibited a continuum of staining intensities between types I and IIA. Using electrophoretic analyses, it was revealed that type I fibres contained exclusively slow myosin heavy chain (HCI), whereas type IIA fibres contained only fast-myosin heavy chain (HCIIa). The C fibre population was characterized by the coexistence of both heavy chains in varying ratios, type IC with a predominance of HCI and type IIC with HCIIa (Table 2). They

concluded that a correlation exists between the myosin heavy chain composition and the histochemical myosin ATPase staining activity.

In a similar study, Staron and Pette (1987b), employed histochemical and electrophoretic techniques on single fibres of the rabbit tibialis-anterior muscle. Histochemically the muscle was comprised of four distinct fibre types (I, IIC, IIA, and IIB). Once again type I fibres contained HCI, and types IIA and IIB contained HCIIa and HCIIb, respectively. A small fraction of fibres (IIAB), that were histochemically intermediate between types IIA and IIb, displayed a coexistence of HCIIa and HCIIb (Table 2).

A study completed by Schiaffino et al. (1989) identified three subpopulations of type II fibres which were classified as type IIa, type IIb, and type IIX. The type IIX fibres were identified using immunocytochemistry, electrophoresis, and myosin ATPase histochemistry (Schiaffino, et al., 1989). Type IIX fibres were unique with respect to histochemical myosin ATPase staining, in that they stained like type IIa fibres after formaldehyde-alkali pretreatment, but reacted like type IIb fibres after preincubation at pH 4.6. However, the type 2X fibers displayed moderate to strong succinate dehydrogenase activity and were similar to type IIa fibres with respect to oxidative potential. (Schiaffino, et al., 1989) (Table 2).

A more recent study, which employed immunoblotting techniques, identified in the rat soleus and extensor digitorum longus muscles, the presence of four different myosin heavy chains (MCH) (Ausoni,

Gorza, Schiaffino, Gundersen & Lomo; 1990). The chronically stimulated muscle was characterized by one type I-MHC, and three subtypes of type II myosin heavy chain; type IIa-, type IIb-, and type IIX-MHC.

Standard histochemistry, in combination with 2D electrophoretic analyses (Pette, 1984; Staron & Pette, 1987a; Termin, Staron & Pette, 1989), and immunohistochemistry (Kucera, Walro, & Gorza, 1992; Schiaffino et al., 1989) have also been employed to differentiate fibre types and resulted in the identification of a continuum of fibre types between type I and type IIb fibres (Table 2).

TABLE 2: Fibre classification based on myosin ATPase activity and additional analyses as indicated

Analyses	Fibre Type							Muscle (s)	Species	References
	I			IIA	IIC	IIAB	IIB	vastus lateralis	human	1,2
	I (soleus)			IIA1		IIA2	IIA3	soleus, plantaris vastus lateralis	rat, rabbit and human	3,1,4
	I (plantaris)									5
electrophoresis	I	IC	IIC	IIA	--	--		soleus	rabbit	6,7,8,9
electrophoresis	I	IC	IIC	IIA	--	IIAB	IIB	tibialis anterior	rabbit	6
electrophoresis	I	IC	IIC	IIA		2D	IIB	EDL and soleus	rat	10
	I		IIC	IIA		IIBτ[n]	IIB»[2]	vastus lateralis	human	11
immunocyto-chemistry	I	IC	2C	2A		2X	2B	soleus	rat	12,13
electrophoresis and immuncyto-chemistry	I			2A		2X	2B	soleus	rat	14
electrophoresis	I	IIC	IIAC	IIA		IIAB	IIB	vastus lateralis	human	15

[n] IIBτ acid labile fibre [2] IIB» acid stable fibre

[1]Lexell, Henriksson-Larsen, Winbald, and Sjostrum (1983); [2]Staron, Hikida, and Hagerman (1983); [3]Gollnick, Parsons, and Oakley (1983); [4]Lexell, Downham, and Sjostrum (1986); [5]Matoba, Allen, Bayly, Oakley, and Gollnick (1985); [6]Maier, Gorza, Schiaffino, and Pette (1988); [7]Staron and Pette (1986); [8]Staron and Pette (1987a); [9]Staron, and Pette (1987b); [10]Termin, Staron, and Pette (1989); [11]Timson (1982); [12]Gorza (1990); [13]Kucera, Walro, and Gorza (1992); [14]Schiaffino, et al., (1989); [15]Staron and Hikida (1992).

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Structural and functional properties of skeletal muscle such as, speed of contraction, enzyme capacities, enzyme histochemical features, demands mediated through the nerve, vascular supply, and hormones, can change under physiological stress (Sjostrom, Downham & Lexell, 1986; Staron, Hikida, & Hagerman, 1983; Houston, 1978). Techniques which have been used to produce this stress on a target muscle and result in the transformation of muscle fibre types are: cross-reinnervation (Close, 1969; Barany & Close, 1971), chronic electrical stimulation (Gorza, et al., 1988; Maier, et al., 1988), and compensatory hypertrophy (Roy, et al., 1985; Ianuzzo, Gollnick & Armstrong, 1976).

The method of cross reinnervation involves taking the motor nerve from a typical slow or fast muscle and innervating it to a muscle with opposing contractile characteristics. With stimulation of the muscle, the contractile properties of the innervated muscle have been shown to exhibit similar contractile characteristics as the original muscle (Barany & Close, 1971).

Similar adaptations within skeletal muscle can be exhibited with chronic electrical stimulation. This technique employs the positioning of two electrodes within a muscle and stimulating it with similar motor nerve impulses carried by either slow or fast muscle. After just four weeks of chronically stimulating the fast twitch muscles of the rabbit, Maier, et al. (1988), observed that the number of type I fibres had increased more than fourfold, while only about six percent of the original type IIb fibres remained.

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After 16 weeks of stimulation, the number of type I fibres had increased to 42%. The greatest relative increase occurred in the type I fibres which may be explained on the basis of the greater utilization of type 1 motor units. Varying the amount and frequency of stimulation, Gorza, Gundersen, Lomo, Schiaffino and Westgarrd (1988) demonstrated fibre type transformation in the rat soleus muscle. Muscles stimulated intermittently for two months at 15 Hz showed a higher percentage of type I fibres. In contrast, the muscles stimulated at a frequency of 100 Hz, were characterized by a greater number of type II fibres.

The compensatory hypertrophy model, which involves the removal of the gastrocnemius/soleus group, produces extensive overload on the target muscle and has also been revealed to cause a transformation of fibre types. Ianuzzo, Gollnick, and Armstrong (1976) showed that the compensated soleus muscle consisted of nearly 100% slow twitch fibres compared to 83% for the control soleus muscle. Furthermore, Roy et al. (1985) observed, after 12 to 14 weeks of overloading a fast twitch muscle, that it also demonstrated greater homogeneity with respect to oxidative characteristics in comparison to the normal soleus muscle, which was comprised of 16% fast-twitch fibres. Also, glycolytic enzyme activity assessed by phosphofructokinase and alpha-glycerophosphate dehydrogenase (α-GPDH) stains had decreased in the compensated rat

soleus muscle in contrast to the normal rat soleus muscle.

The introduction of the needle biopsy technique by Bergstrom (1962) has permitted over the past several decades the classification of human striated muscle into different fibre types. Analyses of cross-sections of human muscle have shown that the proportion of fibres with different properties (type I and type II) varies systematically within a muscle (Lexell, Downham, & Sjostrom, 1986). It was also revealed that the distribution of different fibre types appeared to be uneven within single fascicles. The proportions of type I fibres on the boundaries of the fascicle was usually less than that noted internally (Lexell, Downham, & Sjostrom, 1986; Sjostrom, Downham, & Lexell, 1986).

Lexell, Taylor, and Sjostrom (1985) completed a study to determine the extent of sampling errors associated with the biopsy techniques. Because of the large variability in the proportion of fibre types within a whole muscle, a single biopsy is a poor estimation of the fibre type proportion for a whole muscle. It was recommended that if only one biopsy was going to be completed that the counting of all fibres is of greater benefit compared with counting only half of the fibre number. Also, each sample should contain at least six hundred fibres to substantially reduce the sampling error.

TABLE 3: Histochemical fibre type distribution in the vastus lateralis muscle of man before and after longtitudinal training studies.

Type of Training		Fibre Types I	IIA	IIB	References
8 weeks aerobic endurance training	before	41	37	19	1
	after	43	42*	14*	
24 weeks cross-country running	before	58	26	9.2	2
	after	57	32*	3.4*	
6 weeks aerobic endurance training	before	50	37	12.4	3
	after	56*	34	9.6*	
15 weeks mixed aerobic continuous and interval work	before	41	42	17	4
	after	47*	42	11*	
8 weeks high intensity aerobic work	before	49	38.3	12.8	5
	after	48.8	43.3	8.0*	

* significant at $p < 0.05$

[1]Anderson and Henriksson (1977); [2]Ingjer (1979); [3]Howard (1985); [4]Simoneau, Lortie, Boulay, Marcotte, Thibault, and Bouchard (1985); [5]Baumann, Jagg, Soland, Howard, and Schanb (1987).

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The alteration in fibre types and metabolic potential in response to the stress of exercise have been shown to be the result of the variation in neuromuscular recruitment patterns (Green et al., 1991). Endurance type training adaptive changes will produce a fast-to-slow fibre type transformation (Table 3) and ultimately result in the predominant expression of slow myosin light and heavy chains (Alway, et al., 1988; Rusko et al., 1991; Green, Thomson, Daub, Houston & Ranney, 1991; Maier, et al., 1988). Saltin et al. (1977) demonstrated a difference in metabolic potentials between sedentary and endurance trained subjects. In this study, the oxidative capacities of the type IIa and type IIb fibres in the endurance trained individuals markedly surpassed the potential of type I fibres of untrained subjects.

A study completed by Baumann, Jaggi, Soland, Howald, and Schaub (1987) reported that following many years of intensive endurance training, human skeletal muscle was marked by a high proportion of type I fibres. Their research also revealed that after eight weeks of high intensity endurance training, the type IIb fibre population decreased by 70% in the functioning muscle. Several years of this form of training resulted in a significant reduction of type IIb fibres and an 80% increase in type I and IIa fibres as compared to the sedentary control group. Furthermore, following several years of endurance training, the myosin heavy chain (MHC) associated with type IIa fibres was altered, as evidenced by the presence of new peptide patterns, which resembled

the digestion pattern of slow MHC in fibres which still classified histochemically as type IIa. Therefore, in spite of the presence of the slow isoform of the MHC, these fibres were still classified as type IIa following conventional histochemical treatment (Baumann, Jaggi, Soland, Howald & Schaub, 1987).

A pre and post-season muscle sample was obtained from elite hockey players in a study conducted by Green, Thomson, Daub, Houston, and Ranney (1979). The researchers reported that there was no significant difference in the number of type I fibres. However, within the type II subgroups, a reduction in the type IIb and a significant increase in the type IIa fibre population was observed in the post-season biopsy sample. The type IIb fibres decreased from 12.2% to 3.9% while type IIa fibres increased from 38.0% to 45.2% (Green et al., 1979). Ingjer (1979) conducted a study in which seven young females participated in a 24 week, high intensity endurance training program and reported results which were similar to that of Green et al., (1979). Type IIa fibres increased from 26.4 to 31.5% while type IIb decreased from 9.2 to 3.4% and type IIc fibres increased from .4 to 2.2%. In addition, the endurance trained muscle was characterized by a novel fast fibre, IIAB, which represented a transitional fibre, intermediate between type IIa and type IIb fibres (Ingjer, 1979).

A slight decrease in the number of type I fibers was reported by Sjostrom, Angquist, Bylund, Friden, Gustavsson, and Schersten (1982) following a training program which consisted of running 4

kilometres twice a week and playing basketball once a week. The researchers reported a slight decrease in the number of type I fibres, from 36.5% to 31%, after six months of training. Also, fibres which exhibited a staining pattern different from the typical type IIa or type IIb fibres were present.

Following an analysis of muscle biopsies from strength-trained women, Staron and Hikida (1992), identified, based on the myosin heavy and light chain composition, transitional fibres which in all likelihood represented a continuum of the type C fibre population. The type C fibres were histochemically subdivided into three main fibre types: IC, IIC, and IIAC. The IC fibres were histochemically more similar to the typical type I fibre, and the IIAC were more similar to the type IIa. The oxidative potential of the C fibre type population revealed similarities between fibre type I and fibre type IIa.

Using the formate-KCl buffer as the preincubation medium (pH 4.6) this study will determine if the number of transitional or intermediate fibre types are more predominate in decade trained endurance athletes as compared to sedentary individuals. Also using the traditional stains of Wattenberg and Leong (1960) and Novikoff, Schin, and Drucker (1961) the glycolytic and oxidative capacities of the differentiated fibre types will be compared between the two groups.

CHAPTER III

METHODS AND MATERIALS

Subjects

Thirteen male subjects, seven endurance trained and six sedentary, volunteered for the study after being informed in detail of the nature of the experiment and the potential risks involved. The age, height and weight for each subject were obtained. The trained group had been training aerobically for over a decade at a national level while the other six were considered sedentary individuals. The study was approved by the Ethics Advisory Committee on Human Experimentation at Lakehead University.

Methods

A deep muscle sample was acquired from the lateral aspect of the vastus lateralis, 15 cm above the upper margin of the patella employing the needle biopsy technique of Bergstrom (1962). Samples of muscle were oriented on a cork surface, fixed with OCT (Ames Tissue-Tek) and immediately frozen in 2-methyl butane pre-cooled by liquid nitrogen. The frozen samples were stored at -80°C until analyzed. Prior to the histochemical analysis serial sections 12μm in thickness were cut in a cryostat at -16°C, mounted on cover slips, and allowed to dry at room temperature over night. A minimum of three series of sequential sections were set aside for staining.

Fibres were classified into types based upon differences in

staining intensity for myofibrillar actomyosin ATPase following preincubation at alkaline (pH 9.9) (Padykula & Herman, 1955), with acidic (pH 4.35 or 4.65) (Brooke & Kaiser, 1970), and with a formate-KCl buffer (pH 4.54) (Matoba and Gollnick, 1984; Thayer and Fanti, 1992). Alkaline preincubation was for 10 min at 37°C in a 50 mM glycine buffer containing 18 mM $CaCl_2$, 50 mM NaCl, and 0.1 N NaOH. The pH was adjusted with 4 N HCl. Acid preincubation was performed at pH 4.35 and 4.65 for 90 seconds at 25°C in a sodium acetate (anhydrous) buffer containing 100 mM KCl. The pH was adjusted with concentrated acetic acid. Following the alkaline and acid preincubations the sections were rinsed five times in distilled water. The pretreated sections were then incubated for 30 min in a glycine buffer (pH 9.9) containing 3.1 mM ATP, 50 mM NaCl, 18 mM $CaCl_2$ and 0.1 N NaOH with the temperature maintained at 37°C in a shaking water bath. Sequential activation of the myofibrillar actomyosin ATPase of type I fibres and inactivation of type II fibres was initiated by preincubation at 25°C in a 100 mM formate buffer (pH 4.54) containing 100mM KCl (Gollnick and Matoba, 1984). The pH of the formate-KCl buffer was adjusted by KOH. During the acid preincubations, the sections were removed sequentially from the solutions at pre-determined intervals (10s, 40s, 1.5, 2.5 and 4.0 minutes). The tissue sections were then incubated for 30 min in 100 mM AMpro buffer (pH 9.9) comprised of 3.1 mM ATP, 18 mM $CaCl_2$, and 50 mM KCl with the temperature maintained at 37°C in an Orbit Shak-R-Bath. Prior to exposure to

the muscle tissue, the incubation solution was pre-warmed to 37°C and added to prewarmed jars containing the tissue sections. Following the incubation period, the sections were rinsed five times with water, soaked for 3 min in 1% $CaCl_2$, the water rinse cycle repeated, soaked for 3 min in 2% $CoCl_2$, the water rinse repeated, soaked in 1% $(NH_4)_2S$ for 1 min, and rinsed generously with water. The muscle sections were then dehydrated with alcohol, rinsed with histoclear, and mounted with Permount.

Differences in the oxidative and glycolytic capacities of the fibres were determined following treatment with nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) (Novikoff, Schin, and Drucker, 1961) and alpha-glycerophosphate dehydrogenase (α-GPDH) (Wattenberg and Leong, 1960) respectively.

The pH of all solutions was determined at the temperature outlined in the methodology. Two Fisher Accumet Model 750 pH meters were employed; one calibrated between pH 7.0-10.0 at 37°C whereas the second pH meter was calibrated between pH 4.0-7.0 at 25°C. Both electrodes were checked periodically to ensure response to the manufacturer's specifications. The incubation solutions were made fresh daily.

The staining intensity of the myofibrillar actomyosin ATPase was determined by employing the JAVA image analysis program (Jandel Scientific). The video system was set to translate intensity into numerical data (scale, 1-4 from light to dark).

Fibre counting was completed using a Zeiss Photomicro Jandel

Scientific Java microscope and television screen. The muscle samples, while viewed on the television screen, were divided into different fascicles. All of the fascicles counted were summed to obtain the total number of muscle cells per sample.

Statistical Analysis

For each fibre type a one-way analyses of variance (ANOVA) was used to measure any difference between sedentary and endurance trained subjects. Also a one-way anova was used for oxidative and glycolytic capacities. The means were compared by a Student-Newman-Keuls test.

CHAPTER IV

RESULTS

The mean age of the endurance trained group (n=7) was 35.57 years (±4.99), their mean height was 178.31 cm (±5.31) and their mean weight was 70 kg (5.82). The mean age of the sedentary group (n=6) was 36 years (±8.49), their mean height was 180.34 cm (±8.5), and their mean weight was 85.36 kg (±15.01). All of the physical characteristics of the subjects are listed in Table 4.

Table 4: Descriptive statistics of physical characteristics of both endurance trained and sedentary subjects.

TRAINED (n=7)

Variable	Mean	S.D.	Minimum	Maximum
Age	35.57	±4.99	30	45
Height (cm)	178.31	±5.31	168	185.42
Weight (kg)	70.84	±5.82	62.60	77.11

SEDENTARY (n=6)

Variable	Mean	S.D.	Minimum	Maximum
Age	36	±8.49	27	49
Height (cm)	180.34	±8.5	167.64	190.5
Weight (kg)	85.36	±15.01	68.04	108.86

TRANSITIONAL FIBRES

Histochemically stained serial sections, following alkaline preincubation (pH 9.9) (Padykula & Herman, 1955) and acid preincubation (pH 4.3 or 4.6) (Brooke & Kaiser, 1970), resulted in the initial designation of fibre types into categories of type I, IIa and IIb (Table 5).

Alkaline preincubation (pH 9.9) produced the typical dichotomous staining pattern of dark fibres (type II) with intensity rating of 3.9 and light stained fibres (type I) with a rating of 1.7 (Table 5). The standard acetate buffer (Brooke and Kaiser, 1970) at pH 4.3, produced activation of myosin ATPase activity in the type I fibres (2.8) and an inactivation in the type IIa and type IIb fibre (1.4) population (Table 5). However at pH 4.6, type IIa (1.2) and type IIb fibres (2.3) (Table 5), were clearly distinguishable from each other due to the greater acid lability of the type IIa fibres. The acid stable type I fibres maintained their dark staining intensity (3.8) at this pH.

When muscle sections were preincubated in a formate-KCl buffer (pH 4.6) for various periods there was a progressive activation of type I fibres to a rating of 3 and a sequential inactivation of type IIa and IIb fibres to a rating of 1.4 over the time course examined (Table 5). The type I fibres, with preincubation periods of 30 seconds (sec) and 60 sec were inactivated and stained lightly to intensities of 1.6 and 1.8 respectively, and compared favourably to the rating of 1.7 for type I fibres pretreated at pH 9.9 (Table 5). With the same preincubation times (30 sec and 60 sec), the

TRANSITIONAL FIBRES

type IIa and IIb fibres were easily differentiated on the basis of intensity ratings of 2.8 (type IIa) and 4 (type IIb) and 2.9 (type IIa) and 3.9 (type IIb) respectively (Table 5). The more acid labile type IIa fibre was readily identified at 30 sec of preincubation (2.8).

Acid preincubation for 120 sec produced a staining intensity of the type I fibres similar to that observed after pretreatment in acetate buffer at pH 4.6. During the same time period, the type IIa fibres were lightly stained (1.5) and were similar to the staining intensity observed in acetate buffer at pH 4.3 (1.4) and pH 4.6 (1.2) (Table 5). The type IIb fibres stained dark (4.0) for the same period.

With 180 sec and 240 sec of preincubation in the formate acid pretreatment, there was a progressive darkening of the type I fibres to a rating of 3, whereas the type IIa and IIb fibres became ATPase negative staining lighter at 1.6 and 1.0 and 2.5 and 1.4 respectively (Table 5). As well the staining pattern produced at 240 sec was very similar to that observed at pH 4.3 in acetate buffer (Table 5).

TRANSITIONAL FIBRES

TABLE 5: The formate KCl buffer (pH 4.60) compared to the more conventional staining techniques of Padykula and Herman (1955) and Brooke and Kaiser (1970b).

Fibre types (Staining intensity)*			Preincubation		
I	IIa	IIb	pH	Condition	Time (sec)
1.7±.07	3.9±.17	3.9±.17	9.9	Glycine	600
2.8±.11	1.3±.27	1.4±.40	4.3	Acetate	90
3.8±.09	1.2±.11	2.3±.09	4.6	Acetate	90
1.6±.09	2.8±.06	4.0±.03	4.6	Formate	30
1.8±.04	2.9±.09	3.9±.08	4.6	Formate	60
3.2±.08	1.5±.13	4.0±.04	4.6	Formate	120
3.3±.05	1.6±.09	2.5±.12	4.6	Formate	180
3.0±.10	1.0±.09	1.4±.07	4.6	Formate	240

Values are means ± SEM. *Staining intensity scale (1 = light; 4 = dark).

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In the present study the alkaline preincubation (pH 9.9) produced a staining intensity (1.6) of the type I fibres from the decade trained group (DT), and (2.1) from the sedentary group (NT). The type II fibres had an intensity rating of (3.9) in both the DT and NT groups (Table 6, Appendix C). Similar results were obtained using the formate KCl buffer (pH 4.54) with a preincubation time of 10 sec. The type I fibres were inactivated and stained lightly to intensities of (1.6) DT and (2.1) NT. All the type II fibres were highly activated to intensities of (3.9) in both the DT and NT groups (Fig. 1-2 and 2-2; Table 6, Appendix C).

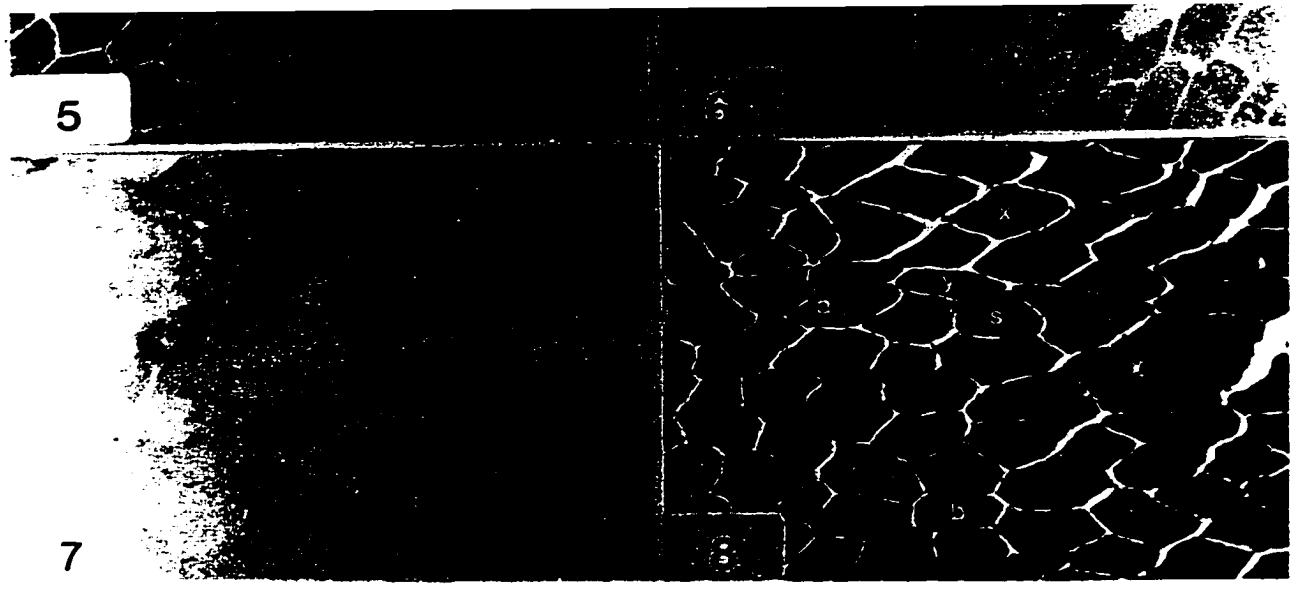

Figure 1: Histochemically stained muscle fibres from highly trained endurance subjects: s-type I, a-type IIa, b-type IIb, x-transitional type IIbaL, y-transitional type IIbaS; 1) alkaline pH 9.9 (Padykula & Herman, 1955); 2) Formate-KCl buffer (pH 4.54) at 10 seconds (Matoba & Gollnick, 1984; Thayer & Fanti, 1992); 3) Formate-KCl buffer at 40 seconds; 4) Formate-KCl buffer at 1.5 minutes; 5) Formate-KCl buffer at 2.5 minutes; 6) Formate-KCl buffer at 4 minutes; 7) α-GPD (Wattenberg & Leong, 1960); 8) NADH-TR (Novikoff, Schin, & Drucker, 1961)

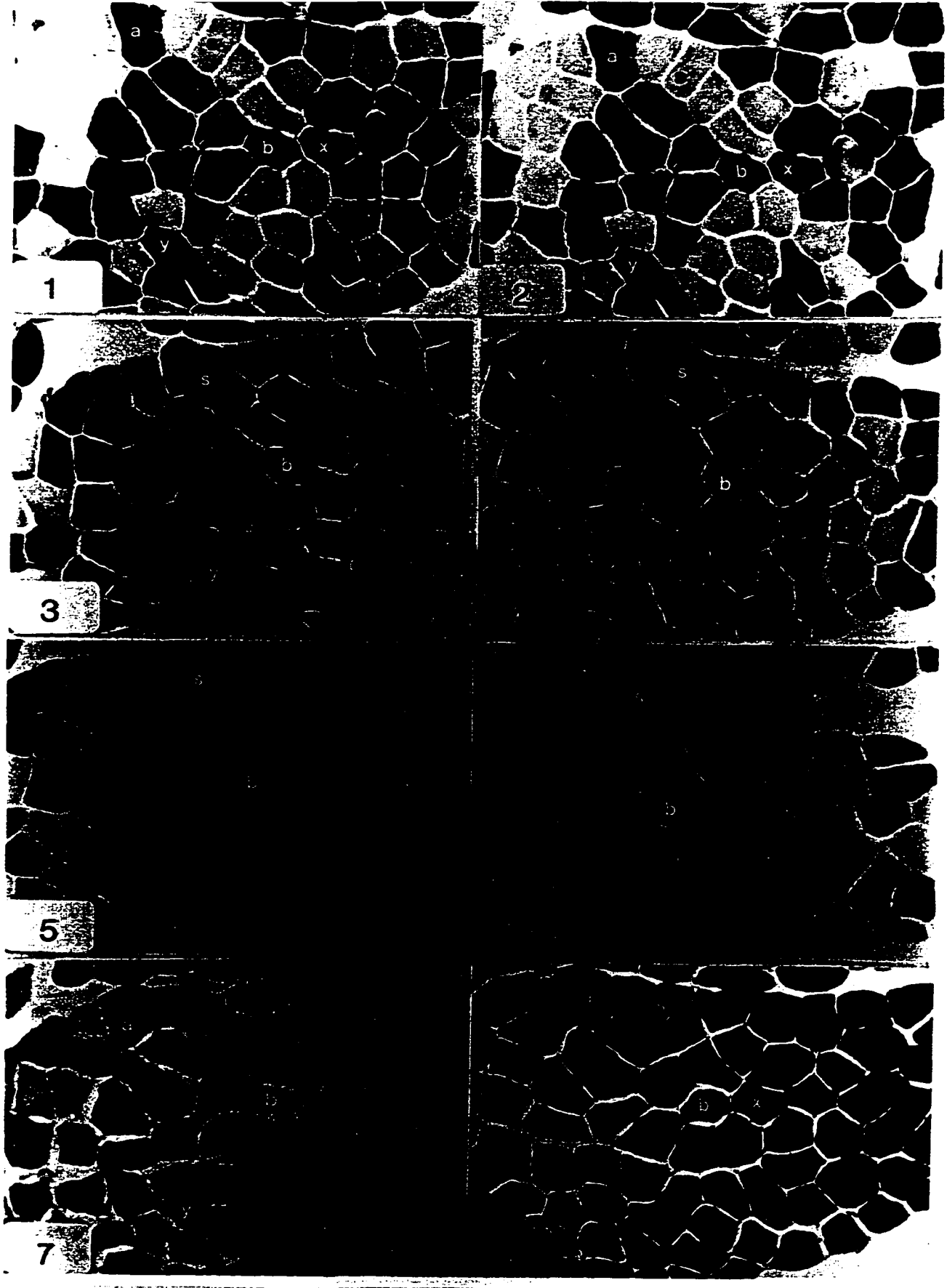

Figure 2: Histochemically stained muscle fibres from sedentary subjects: s-type I, a-type IIa, b-type IIb, x-transitional type IIbaL, y-transitional type IIbaS; 1) alkaline pH 9.9 (Padykula & Herman, 1955); 2) Formate-KCl buffer (pH 4.54) at 10 seconds (Matoba & Gollnick, 1984; Thayer & Fanti, 1992); 3) Formate-KCl buffer at 40 seconds; 4) Formate-KCl buffer at 1.5 minutes; 5) Formate-KCl buffer at 2.5 minutes; 6) Formate-KCl buffer at 4 minutes; 7) α-GPD (Wattenberg & Leong, 1960); 8) NADH-TR (Novikoff, Schin, & Drucker, 1961)

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As the preincubation time was increased, the myosin ATPase activity of the type I fibres became more activated, as evidenced by the darker staining intensity in both the DT and NT subjects (Fig 1 and 2). However, at 40 sec the formate KCl buffer (pH 4.54) delineated at least four staining intensities of the type II fibres, IIa, IIb, IIbaL, and IIbaS (Table 6; Appendix C). The preincubation at 40 sec demonstrated similar staining patterns as the type IIa and type IIb fibres identified following acidic preincubation at pH 4.35 or 4.65 (Brooke & Kaiser, 1970b) (Table 5). Type IIa fibres were lightly stained for both the DT group and the NT group. Type IIb fibres were the most activated, displaying intensities of (2.6) in the DT group and (3.6) in the NT group. However, one other staining intensity was observed at 40 sec in the DT group using the formate KCl (pH 4.54). This uncharacteristic staining intensity, (1.9) at 40 sec, will be refereed to as a type IIbaL (more acid labile) transitional/intermediate fibre type. Furthermore, at 40 sec the NT group demonstrated two other staining intensities which differed from the type II fibres. These different staining intensities will be refereed to as type IIbaL (2.3) and type IIbaS (3.0) transitional/intermediate fibres (Fig 2-3).

It was not until 90 sec of preincubation, in the formate KCl buffer (pH 4.54), that the transitional/intermediate type IIbaS fibre appeared in the DT group staining (2.0) while the transitional/intermediate type IIbaL stained (1.2), type IIb fibres

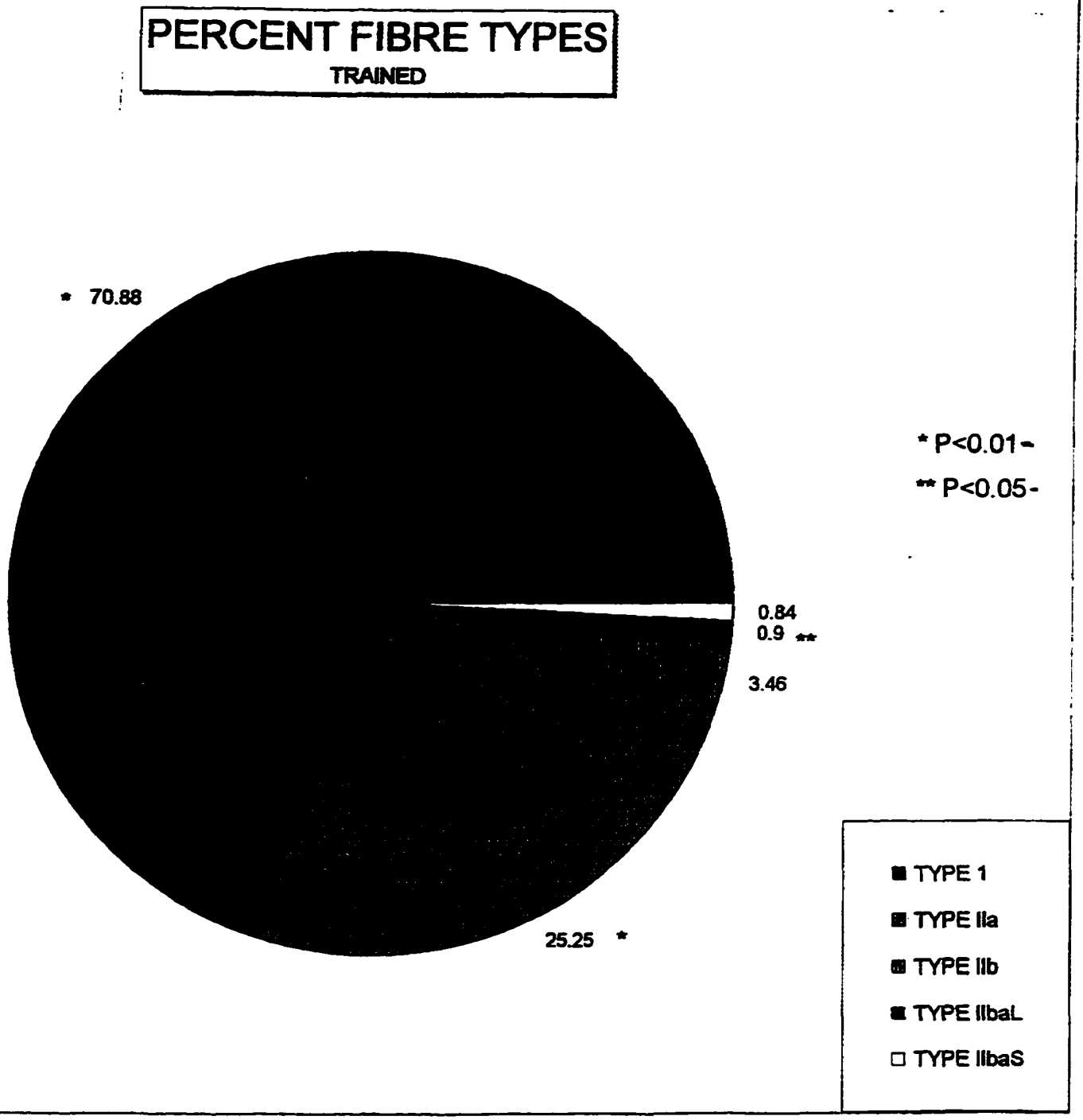
(1.3) and type IIa fibres (1.0) (Fig 1-4). The transitional intermediate fibre type IIbaS continued to remain more stable throughout the preincubation sequence of the DT group (2.0 at 150 sec and 2.1 at 240 sec) as compared to the type IIbaL and type IIb fibres (1.0 at 150 and 240 sec). The IIbaS fibre stained 2.3 at 150 sec and 1.8 at 240 sec in the NT group which was darker as compared to the type IIbaL (1.8 at 150 and 240 sec) and type IIb (2.9 at 150 and 240 sec) fibres for the same preincubation time (Fig 2).

In the present study, the rare type IIc fibre was identified on the basis of the relative stability of myofibrillar actomyosin ATPase following exposure to both alkaline and acid preincubation (Brooke and Kaiser, 1970b).

There was a very noticeable difference in the muscle fibre composition between the endurance-trained athletes as compared to the sedentary individuals (Figure 3 and 4). The decade trained athletes who participated in endurance type events revealed in their histochemical profile a preponderance of type I fibres (70.88% ± 9.77) as compared to the sedentary group (37.73 ± 20.89) ($P < 0.01$) (Figures 3 and 4). As a result of the greater number of type I fibres in the decade trained muscle, the quantity of type IIa fibres in sedentary subjects differed significantly (51.84% ± 18.12) ($P < 0.01$) from the decade trained subjects (25.25% ± 10.41) (Figures 3 and 4). The number of transitional/intermediate type IIbaL was also greater ($P < 0.05$)

TRANSITIONAL FIBRES

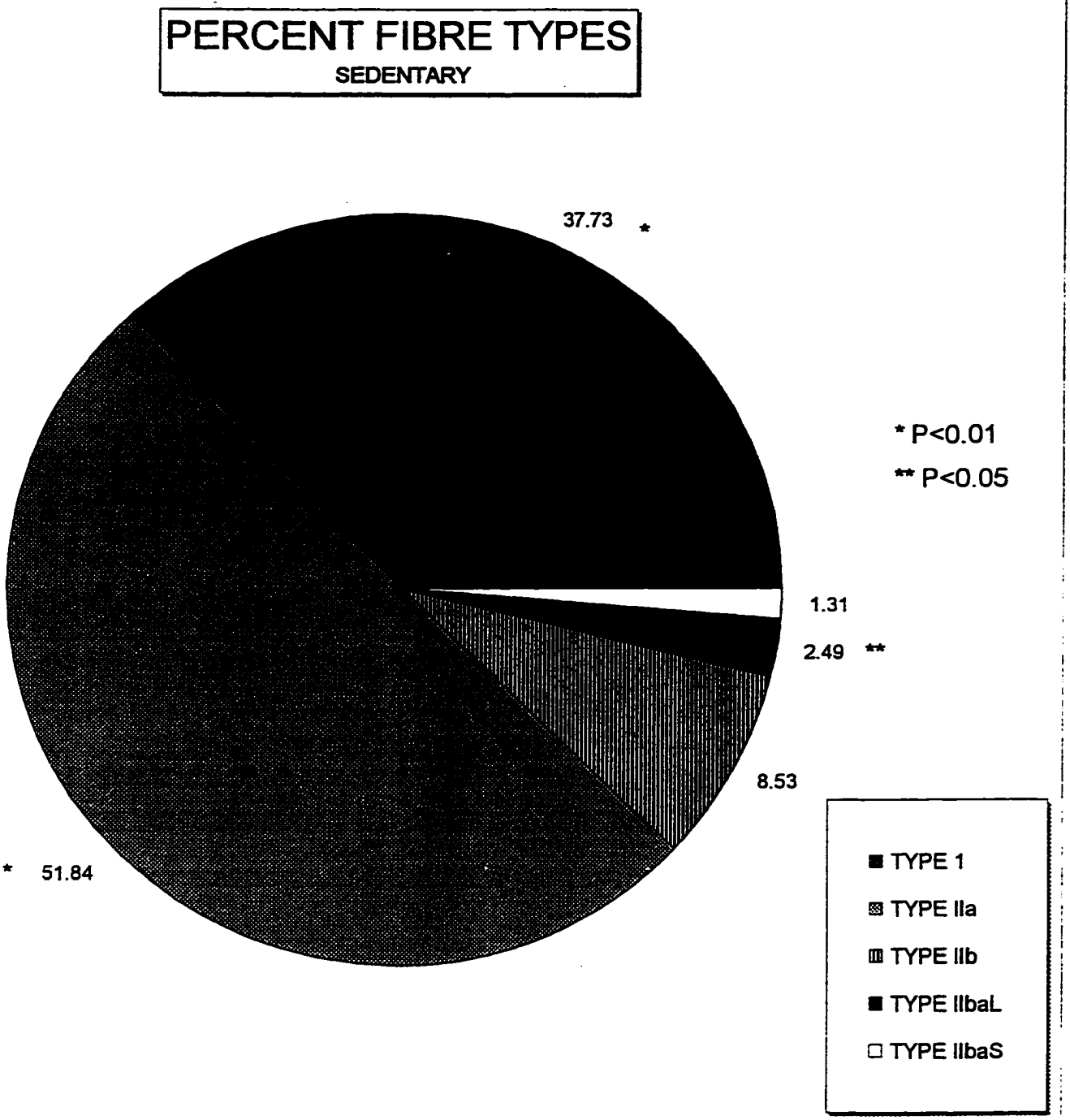
in the sedentary group (2.48% ± 1.19) as compared to the decade trained subjects (0.90 ± 0.86) (Figures 3 and 4).



STANDARD DEVIATIONS

TYPE I	TYPE IIa	TYPE IIb	TYPE IIbaL	TYPE IIbaS
± 9.77	± 10.41	± 2.43	± 0.86	± 0.99

Figure 3: Percentage of fibre types in highly trained endurance athletes. Significant difference when compared to fibre type in sedentary subjects.



STANDARD DEVIATIONS

TYPE 1	TYPE IIa	TYPE IIb	TYPE IIbaL	TYPE IIbaS
± 20.89	± 18.12	± 5.97	± 1.19	± 1.07

Figure 4: Percentage of fibre types in sedentary subjects. Significant difference refers to comparison of fibre type with Trained.

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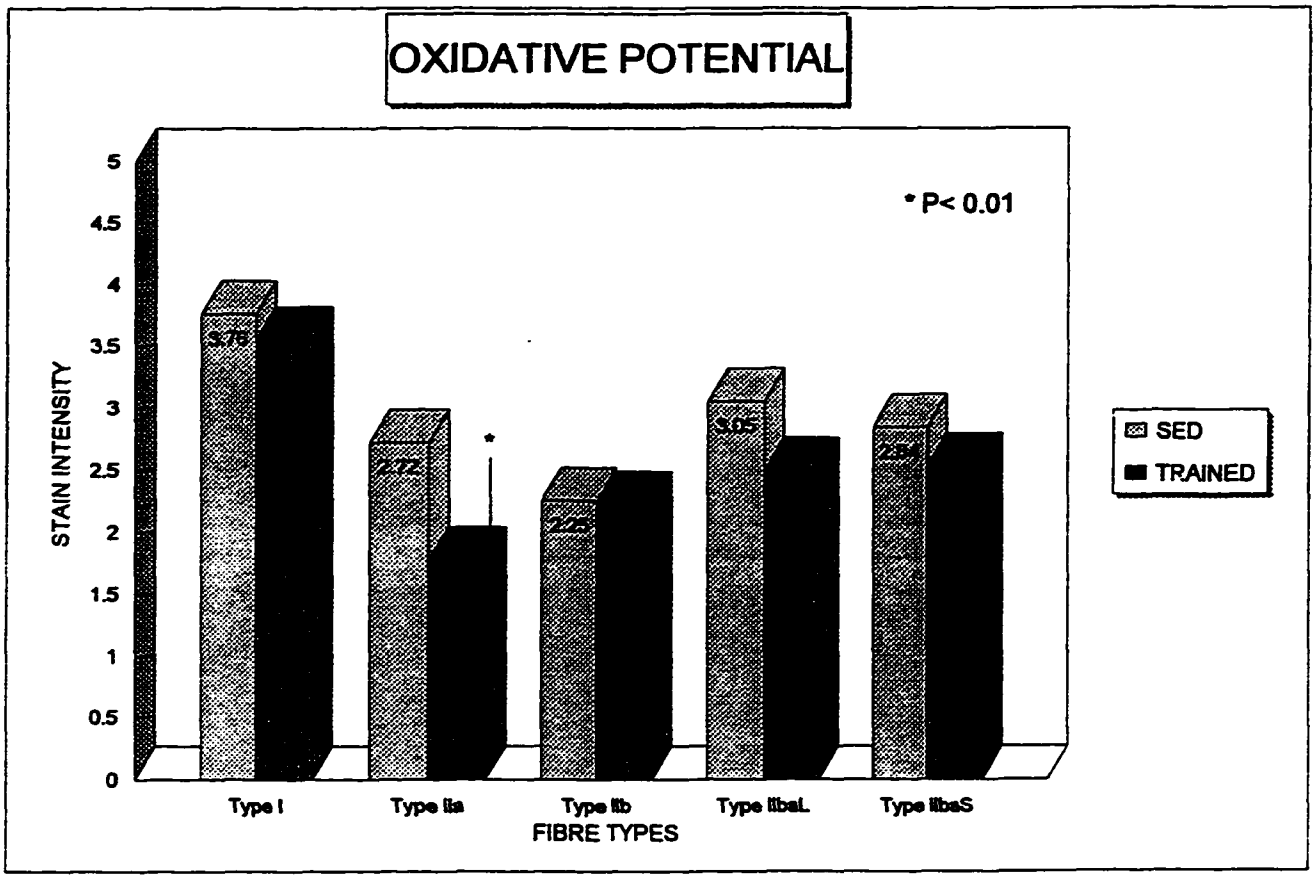
Oxidative potential, as determined by the NADH-TR stain (Figure 1-8 and 2-8), was consistently high in type I fibres in both the decade trained and sedentary subjects. The type IIa fibres from the sedentary group demonstrated a significantly greater oxidative capacity ($P < 0.01$) than the type IIa fibres of the decaded trained subjects (Figure 5).

The use of the α-GPDH stain to determine glycolytic differences between fibre types revealed, as expected, a darker staining intensity with the type II fibres as compared to the type I fibres (Fig 1-7, 2-7). Although no significant differences were observed, the type I fibres of the sedentary group stained darker than the type I fibres of the decade trained (Figure 6). Using the α-GPDH stain, the transitional/intermediate type IIbaL fibres of the sedentary group revealed lower glycolytic potential as compared to the transitional/intermediate type IIbaL fibres from the decade trained group. Also, following exposure to the α-GPDH stain, the transitional/intermediate type IIbaS fibres of the sedentary group revealed slightly higher glycolytic potential as compared to the transitional/intermediate type IIbaS fibres from the decade trained subjects (Figure 6).

TRANSITIONAL FIBRES

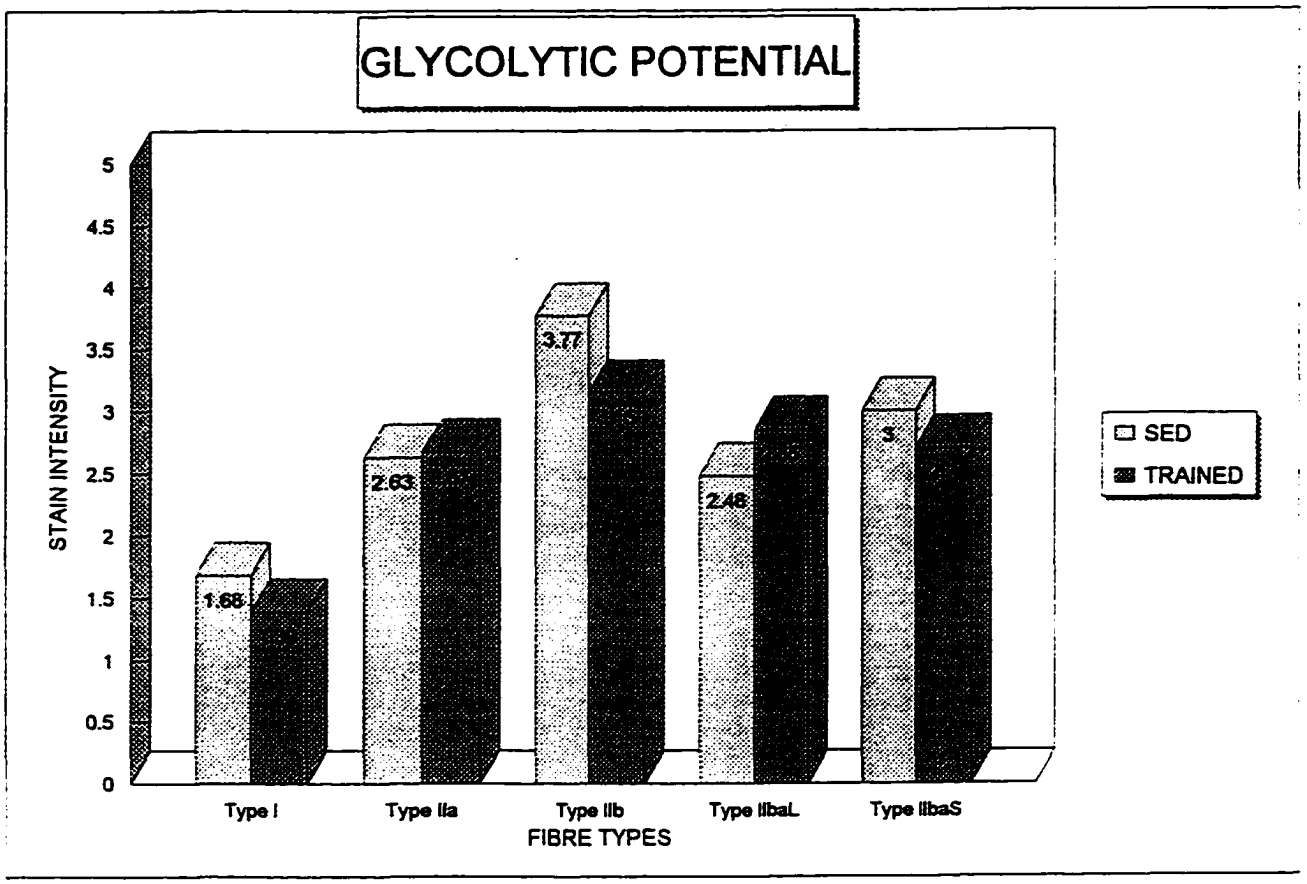


STANDARD DEVIATIONS

	Type I	Type IIa	Type IIb	Type IIbaL	Type IIbaS
SEDENTARY	± 0.19	± 0.50	± 0.40	± 0.30	± 0.37
TRAINED	± 0.14	± 0.67	± 0.92	± 0.66	± 0.40

Figure 5: Oxidative potential of different fibres between highly trained endurance and sedentary subjects

TRANSITIONAL FIBRES



	STANDARD DEVIATIONS				
	Type I	Type IIa	Type IIb	Type IIbaL	Type IIbaS
SEDENTARY	± 0.45	± 0.35	± 0.21	± 0.79	± 0.70
TRAINED	± 0.32	± 0.51	± 0.53	± 0.42	± 0.13

Figure 6: Glycolytic potential of different fibres between highly trained endurance and sedentary subjects

CHAPTER V

DISCUSSION

The present study produced results similar to the preliminary work completed by Thayer and Fanti (1992) which revealed that the histochemical method using the formate KCl buffer (pH 4.60) as the preincubation medium was a reliable and time efficient means for the identification of fibre types in human skeletal muscle. Also the present study clearly demonstrated that the number of transitional/intermediate fibres, in both the sedentary and endurance trained subjects, constituted a very small percentage of the total fibre population. The number of type I fibres was significantly higher in the decade trained subjects while the number of type IIa was significantly higher in the sedentary group. As well the oxidative potential of the type IIa fibres in the decade trained group was surprisingly lower when compared to the type IIa fibres from the sedentary subjects.

Histochemical classification of fibre types depends on the sensitivity of the myofibrillar actomyosin ATPase to a variety of factors. Currently, to identify the polymorphic forms of the proteins that comprise the myofibrillar complex, the most commonly employed methods require preincubations in a variety of buffers at pH 9.9, 4.3 and 4.6. The method employed in the present study provides an alternative scheme for the identification of muscle fibre types based on a time dependent differential activation of

the myofibrillar actomyosin ATPase (Gollnick et al., 1983; Matoba et al., 1985).

The present study using the formate-KCl buffer (pH 4.54) based on a time dependent differential activation of the myofibrillar actomyosin ATPase, distinguished four subtypes of the type II fibre population: type IIa, IIb, IIbaL (more acid labile) and IIbaS (more acid stable). The key question can be asked as to how the staining pattern produced by the acid preincubation in formate-KCl corresponds to the more orthodox methods for fibre classification. The type I and four subtypes of the type II fibres identifiable from the time course (10 - 240 sec) of acid inactivation of myofibrillar actomyosin ATPase were the same as the type I, IIa, IIb, and IIc fibres distinguished after preincubation at pH 9.9, 4.3 and 4.6. (Table 5).

Acid pretreatment (between 0.5 and 5 min) of rat skeletal muscle in an acetate or citrate buffer provides reproducible results with activation of type I and differential inactivation of three type II fibre sub populations designated as A1, A2, and A3 (Gollnick et al., 1983). Similar results in human muscle tissue were obtained after acid pretreatment in formate KCl at pH 4.6 (Matoba et al., 1985). However, the limited preincubation times which Matoba et al., (1985) used for the sequential inactivation of the fibres, and their failure to compare the results to typical staining patterns produced after standard acid pretreatment (pH 4.3 and 4.6), provided only marginal data as to the efficacy of this

method to identify all types of human muscle tissue. Classification of the type IIA2 fibres was difficult due to their variable staining intensity (Matoba et al., 1985).

In the present study, human muscle tissue sections were preincubated over a predefined time course in the formate KCl buffer and the results were compared to those of Matoba et al. (1985). Changes similar to those of Matoba et al., (1985) required a 4 minute preincubation time and provided for the classification of four distinct fibre types, including the type I fibre and four type II fibre classifications.

The staining patterns produced, with a formate KCl buffer (pH 4.6) (Matoba and Gollnick, 1984), were compared and revealed that it clearly differentiated the same fibres as the alkaline and acid preincubations but also identified three subtypes of the type II fibres. Their research confirmed that the staining pattern produced by acid preincubation in formate-KCl corresponds to the more orthodox methods for fibre classification. The type I and three subtypes of the type II fibres identifiable from the time course (30sec - 240sec) of the acid inactivation of myofibrillar actomyosin ATPase were the same as the type I, type IIa, IIb and IIc fibres distinguished after preincubation at pH 10, 4.3 and 4.6.

The present investigation, comparing the muscle fibre profile of sedentary and decade trained men, employed in a more extensive manner the same histochemical method, used in the preliminary work of Thayer and Fanti (1992). The present study revealed adaptive

changes which produced either a type IIb to type IIa or type IIa to type I fibre transformation (Howard, 1985; Simoneau, Lortie, Boulay, Marcotte, Thibault, & Bouchard, 1985; Baumann, Jaggi, Soland, Howald & Schaub, 1987). The number of type I fibres was significantly greater in the decade trained group as compared to the sedentary group. These findings are similar to Simoneau et al., (1985), and Howard (1985) who used a pre and post longitudinal training study and reported that the number of type I fibres increased significantly after six to 15 weeks of aerobic endurance training. Baumann et.al. (1987), detected some training-induced changes in the peptide pattern of the myosin heavy chains in the type IIa fibres which marked the beginning of their transition towards the slow type I fibres. Due to the research design employed in the present investigation it is not possible to conclude, in a definitive way, that endurance training has produced a transformation from type II to type I. However, based on previous research (Baumann et al., 1987; Simoneau et al., 1985; Howard, 1985) which employed a pre - post research design, it is highly likely that the larger percentage of type I fibres represents a transformation in fibre type.

In addition, the percentage of type IIa fibres in the sedentary subjects was significantly greater when compared to the decade trained individuals. These findings contradict previous research which demonstrated that with endurance training the number of type IIa fibres increased (Anderson & Henriksson, 1977; Ingjer,

1979; Baumann, Jagg, Soland, Howald, & Schanb, 1987) or at least stayed the same (Simoneau, et al., 1985). These studies were longitudinal in nature and the amount of training was limited to 24 weeks. In all likelihood, in the present study, the high percentage of type I fibres in the decade trained group represents a transformation of type IIa fibres to type I fibres as a result of at least ten years of high intensity aerobic training. The remaining type IIa fibres in the decade trained group may be comprised of a certain percentage of fibres which have transformed from the type IIb fibre population. Anderson and Henriksson (1977) and Staron, Hikida, and Hagerman (1983) have previously shown that there is a gradual transformation from type IIb fibres to type IIa fibres as evidenced by the different ratios of HCIIa and HCIIb following single fibre analysis. Although non-significant, the type IIb fibre population in the decade trained group was much lower in comparison to the sedentary group.

The formate KCl buffer (pH 4.54) not only differentiated the traditional type I, type IIa, type IIb, and the rare type IIc fibres but also revealed the existence of two additional fibre types which produced a staining pattern different from the typical type IIa and IIb fibres. In the present study these transitional/intermediate fibres have been labelled type IIbaL (more acid labile) and type IIbaS (more acid stable).

Transitional or intermediate fibres have been observed in the past, in both rodent and human skeletal muscle, when muscle fibres

TRANSITIONAL FIBRES

transform from one type of fibre to another (Table 2). Using rabbit muscle tissue Staron and Pette (1987a), Staron and Pette (1987b), and Maier, Gorza, Schiaffino, and Pette (1988) revealed on single fibre analysis that each fibre, longitudinally, could contain different polymorphic forms of the proteins that comprise the myofibrillar complex. Maier,et al. (1988) concluded that the stimulated-induced fast-to-slow transformation of the chronically stimulated fast-twitch muscles of the rabbit is a complex process with the coexistence of several myosin heavy chain isoforms in the same fibre. Staron and Pette (1987b) demonstrated, with single fibre analysis on a transforming fibre from a chronically stimulated rabbit muscle, that it not only co-expressed fast and slow myosin subunits but did so nonuniformly along its length. It was concluded from their research that a correlation exists between the myosin heavy chain composition and the histochemical mATPase staining pattern. Type I fibres have been shown to be comprised of slow myosin heavy chain (HCI) and type IIa fibres contained only fast-myosin heavy chain (HCIIa). The slight differences in the staining intensities of the muscle fibres observed in the present study (Figure 2 and 3) revealed that these transitional or intermediate fibres may be comprised of more than one type of myosin heavy chain. A small fraction of fibres (IIAB), histochemically intermediate between types IIa and IIb, displayed a coexistence of HCIIa and HCIIb (Staron & Hikida, 1992; Staron & Pette, 1987b; Lexell et al., 1983; and Staron et al., 1983).

Therefore, the possibility exists that the slight differences in the staining intensities of the transitional or intermediate fibres, observed in the present study, suggest that these fibres maybe comprised of both myosin HCIIa and HCIIb.

However, an alternative possibility is that these intermediate or transitional fibres may be comprised of a unique isoform of the myosin heavy chain (Figure 1). The existence of four different myosin heavy chains (MHC) have been revealed in rat skeletal muscle by Ausoni et al. (1990) and Gorza (1990). The researchers employed immunoblotting techniques and found that the chronically stimulated muscle was characterized by one type I-MHC, and three subtypes of type II myosin; type IIa-, type IIb-, and type IIX-MHC. Similarly, Schiaffino et al. (1989), identified the type IIX fibre type in rat muscle using immunocytochemistry, electrophoresis and myosin ATPase histochemistry. The type IIX fibres were unique with respect to histochemical myosin ATPase staining in that they stained like type IIa fibres after formaldehyde-alkali pretreatment but reacted like type IIb fibres after preincubation at pH 4.6. It is conceivable that the intermediate or transitional fibres revealed in the present investigation may be characterized by a MHC similar to the type IIX-MHC (figure 1). However this is highly speculative as no immunohistochemistry or electrophoresis was carried out in an attempt to identify the IIX-MHC.

Although most researchers have concluded that transitional fibres are a consequence of aerobic training (Ausoni et al., 1990;

Schiaffino et. al., 1989), the present study revealed little differences in the number of transitional or intermediate fibres between the decade trained endurance athletes when compared to the sedentary individuals. This is likely due to the fact that the transformation of type II to type I in the decade trained subjects had occurred well in advance of the muscle biopsies obtained in this study. For example, if muscle biopsy samples had been obtained following 3-5 years of aerobic training, significant differences in transitional or intermediate fibre types from the controls may have been observed. In the sedentary group the mean number of type IIbaL fibres was significantly greater as compared to the decade subjects. However, it should be remembered that this fibre type only comprised 1.19% of the total fibre population in the sedentary group. In addition, differences in their histochemical staining intensity may be due to the presence of either a unique MHC (Ausoni et al., 1990; Schiaffino et al., 1989) or the existence of more than one MCH in a single fibre (Staron & Hikida, 1992; Staron & Pette, 1987b; Lexell et al., 1983; and Staron et al., 1983).

The oxidative stain, (NADH-TR), and the glycolytic stain, (α-GPDH) revealed that the fibre type IIbaL was more similar in staining pattern to the type IIb fibre. However, the type IIbaL fibres stained more intensely with the NADH-TR as compared to the

type IIb fibres. Therefore the IIbaL fibre is similar to the type $2B_L$ fibre type previously identified in the preliminary study conducted by Thayer and Fanti (1992). The type $2B_L$ fibre displayed an oxidative staining intensity intermediate between the type IIa and type IIb fibres (Thayer and Fanti 1992).

The type IIbaS fibre displayed a lower glycolytic potential as compared to the IIbaL fibre type and was similar to the type IIa fibre in both the sedentary subjects and the decade trained subjects. In addition, the type IIbaS fibre stained quite intensely with the NADH-TR stain compared to all other fibres with the exception of the type I fibres. Based on these staining characteristics the IIbaS fibre is similar in it's histochemical characteristics to the type 2X fibre reported by Schiaffino et al. (1989) which displayed moderate to strong oxidative activity and was more similar to the type IIa in this respect. However, it is difficult to make comparisons due to differences between human and rat skeletal muscle in response to histochemical treatment.

In the present investigation, it was not possible to identify subtypes of the type I fibre population as no immunohistochemistry or electrophoretic analysis were conducted. Staron and Pette (1986) used histochemical and electrophoretic techniques on single fibres of rabbit soleus muscle and identified four fibre types: type I, IC, IIC, and IIa. Staron and Hikida (1992) continued to study the C-fibre population in strength-trained women. The C fibre population was characterized by the coexistence of two myosin

heavy chains in varying ratios, type IC with a predominance of HCI and type IIC with HCIIa. Although, in our study, subtle differences in staining intensities for α-GPDH were observed within the type I fibre population (Fig. 2-7 and Fig. 3-7), no differences were revealed following treatment with the myosin ATPase stain.

Differences in the oxidative staining intensity of fibres within the type IIa fibre population have been demonstrated. Previous research has revealed that type IIa and type IIb fibres can adapt to aerobic endurance training by an increase in their oxidative capacity to a level which may surpass the aerobic capacity of type I fibres observed in untrained subjects (Saltin et al., 1977). Surprisingly, this was not observed in the present study, as the oxidative potential of the type IIa fibres from the sedentary group was significantly higher when compared to the type IIa fibres from the decade trained group. With a decade of endurance training it is likely that a large percentage of the type IIa fibres from the highly trained group have transformed to become type I fibres and the majority of the remaining type IIa fibres may represent fibres with a low oxidative capacity as compared to those IIa fibres which have transformed to type I fibres. In addition, some of these low oxidative type IIa fibres may represent IIa fibres which have transformed from the type IIb fibre population. Following a 24 week high intensity endurance training program, Ingjer (1979) reported an increase in the type IIa fibre population concomitant with a decrease in the type IIb fibre population. A

transformation of this kind may partially explain the lower oxidative potential of the type IIa fibres in the decade trained group. This observation is also supported by the differences in the number of type I, type IIa and type IIb fibre types when comparing the decade trained group to the sedentary group. The decade trained group demonstrated a significantly higher percentage of type I fibres and a significantly lower percentage of both type IIa and type IIbaL fibres as compared to the sedentary group.

The oxidative staining intensity of the type I fibres in the decade trained group was similar when compared to the type I fibres in the sedentary group. Perhaps as a result of the manipulation of the traditional myosin ATPase stain, the subtypes of the C fibre population, previously identified by Staron and Pette (1986) and Staron and Pette (1992), could not be detected. It is possible that the similar oxidative potentials of the type I fibres in the sedentary and decade trained group was due, not only to the extensive transfer of fast to slow fibre types, but also to the possible presence of the undetected IC and IIC intermediate fibre types.

In summary, the histochemical method using the formate KCl buffer (pH 4.60) as the preincubation medium was a reliable and time efficient means for the identification of fibre types in human skeletal muscle. It not only identified the traditional fibre types but also identified two transitional or intermediate fibre types. These two fibre types make up a very small percentage of

the total fibre population and in all likelihood represent intermediate fibre types which are not in the process of transforming from fast to slow. The number of type I fibres was significantly higher in the highly trained subjects while the number of type IIa was significantly higher in the sedentary group and these results suggest that the subjects in the trained group were not born with this high percentage of type I fibres, but reflects a transformation of type IIa to type I fibre type. As well this study revealed that the oxidative potential of the type IIa fibres in the endurance trained group was surprisingly lower when compared to the type IIa fibres from the sedentary subjects.

CHAPTER VI

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This study, employing a greater number of subjects, supports the preliminary work by Thayer and Fanti (1992), and demonstrates the efficacy of the formate KCl buffer (ph 4.54) in histochemically identifying fibre types as compared to the more conventional methods of Padykula and Herman (1955) and Brooke and Kaiser (1970b). This investigation also posed the question whether the number of transitional/intermediate fibres were more predominate in decade trained endurance athletes as compared to sedentary individuals, and examined possible aerobic and glycolytic differences in these fibres. Muscle biopsies were obtained from the vastus lateralis muscle of seven decade trained endurance athletes and six sedentary individuals for the purpose of determining the effectiveness of the formate KCl buffer (pH 4.54) in identifying all fibre types in human skeletal muscle. Nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) and alpha-glycerophosphate dehydrogenase (α-GPDH) activity were used to examine the oxidative and glycolytic capacities of all fibre types. For each fibre type a one-way analysis of variance (anova) was used to measure any difference ($p < 0.05$) between sedentary and endurance trained subjects. Also a one-way anova was used for oxidative and glycolytic capacities. The means were compared by a Student-Newman-Keuls test.

TRANSITIONAL FIBRES

Conclusions

The following conclusions can be made based on the research findings:

1. The formate-KCl buffer (pH 4.54) was not only effective in distinguishing the traditional type I, IIa, and IIb fibre types but was also effective in identifying intermediate or transitional fibres, and supports the preliminary work of Thayer and Fanti (1992).

2. The number of transitional/intermediate fibres type IIbaL was more predominate ($P < 0.05$) in the sedentary group (2.48% ± 1.19) as compared to the endurance trained (0.90 ± 0.86).

3. That long term endurance training did not produce a wide spectrum of transitional/intermediate fibre types when the human skeletal muscle was treated with the formate KCl buffer.

4. The athletes engaged in endurance type events demonstrated a significant difference ($p < 0.01$) in the percentage of type I fibres (70.88% ± 9.77) as compared to the sedentary group (37.73 ± 20.89).

5. The number of type IIa fibres was significantly greater ($P < 0.01$) in the sedentary group (51.84% ± 18.12) as compared to the endurance trained group (25.25% ± 10.41).

6. The oxidative staining intensity of the type IIa fibres from the endurance trained group (1.77 ± 0.67) was significantly less ($P < 0.01$) as compared to the sedentary group (2.72 ± 0.50).

TRANSITIONAL FIBRES

Recommendations

1. Application of electrophoretic analyses and immunohistochemistry to examine the MHC composition of the transitional/intermediate fibre types as revealed by standard histochemistry.

APPENDIX A

Consent Form For Participants

SUBJECT ..... DATE ..... TIME .....

1. I agree to participate in an investigation by Drs. ..... and/or such assistants as may be selected by them, to perform the following procedure(s):

   two muscle samples (35 to 50 mg) will be taken from a small 1 cm long incision from the vastus lateralis using a biopsy needle under local anaesthetic (xylocaine hydrochloride). Although a rare occurrence, there exists the possibility of a hypersensitivity reaction to the local anaesthetic. Slight discomfort will persist for 1 to 2 hours following the surgery.

2. I also understand that slight muscle soreness may persist for a few days following surgery and that I may, at any time, refuse to continue and/or begin the experiment and withdraw from participation. The Drs. ..... have assured me of confidentiality and will discuss the results personally with me in the future.

....

Witness | Signature of Subject | Signature of Doctor(s)

APPENDIX B

Letter to Participants
LAKEHEAD UNIVERSITY
SCHOOL OF PHYSICAL EDUCATION
HUMAN PERFORMANCE LABORATORY

Dr. Bob Thayer
343 - 8653

At the present time three distinct fiber types have been identified in human skeletal muscle. However preliminary research at Lakehead University suggests there exists a fourth fiber type, termed $2B_L$. This fast-twitch fiber was demonstrated to possess intermediate oxidative capacity (Thayer & Fanti 1992). In addition our recent work reveals that this $2B_L$ fiber may be more prevalent in highly trained endurance muscle. Therefore based upon this evidence the purpose of the research is to determine the effect of many years (at least a decade) of high-intensity endurance training on the expression of this unique fiber type in human muscle. Each subject's muscle sample will be exposed to a variety of histochemical techniques in order to ascertain fiber profile.

Controls as well as highly trained endurance athletes will be exposed to the muscle biopsy technique. Under local anaesthetic (xylocaine hydrochloride), several small, 35 to 50 mg samples of thigh muscle will be taken using a biopsy needle from a small 1 cm long incision. Although a rare occurence, there exists the possibility of a hypersensitivity reaction to the local anaesthetic. All procedures will be performed by a medical doctor and will take approximately 30 minutes to complete.

The subject may experience slight discomfort following the surgery and this muscle soreness may persist for a period of 24 hours. Each subject may at any time refuse to continue the experiment or withdraw from the study at any time. Furthermore the researchers wish to assure the participants that their identity will remain confidential as no names will be used in the study. The results of the study will be made available to each subject.

I look forward to your participation in the study. Please feel free to contact myself if you require further information.

Sincerely,

Dr. R. Thayer

APPENDIX C

Table 6. TRANSITIONAL FIBRES

Histochemical staining intensities of both highly trained endurance and sedentary subjects using the methods of Padykula and Herman (1955) and the Formate CK1 buffer (pH 4.54) of Matoba and Gollnick (1984).

	FIBRE TYPES				
	TYPE I	TYPE IIa	TYPE IIb	TYPE IIbaL	TYPE IIbaS
SUBJECTS					
TRAINED					
(pH 9.90)[1]	1.6	3.9	3.9	3.9	3.9
Formate KC1 (pH 4.54)[2]					
10 sec	1.6	3.9	3.9	3.9	3.9
40 sec	2.5	1.0	2.6	1.9	2.6
90 sec	2.2	1.0	1.3	1.2	2.0
150 sec	3.1	1.0	1.0	1.0	2.0
240 sec	3.4	1.0	1.0	1.0	2.1
SEDENTARY					
(pH 9.90)[1]	2.1	3.9	3.9	3.9	3.9
Formate KC1 (pH 4.54)[2]					
10 sec	2.1	3.9	3.9	3.9	3.9
40 sec	3.6	2.1	3.6	2.3	3.0
90 sec	3.9	1.7	3.9	2.2	2.9
150 sec	3.9	1.4	2.9	1.8	2.3
240 sec	3.9	1.4	2.9	1.8	1.8

[1]Padykula and Herman (1955), [2]Matoba & Gollnick (1984) Formate KC1

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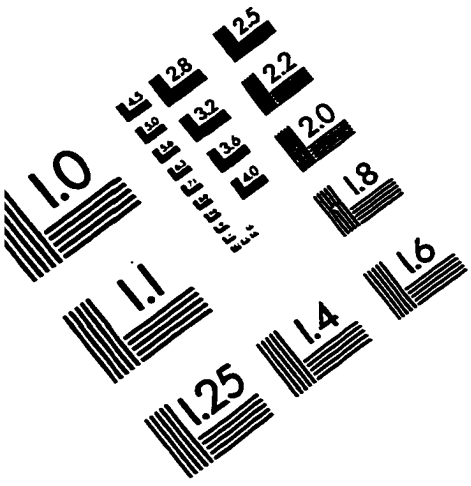
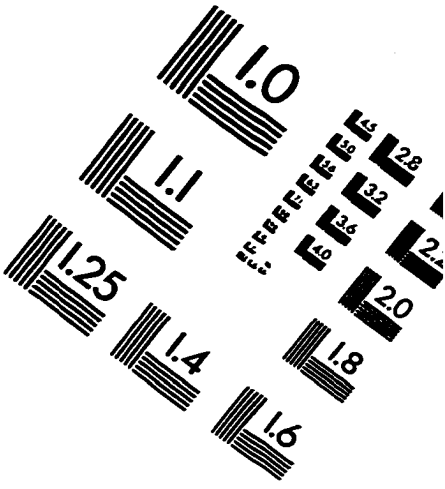
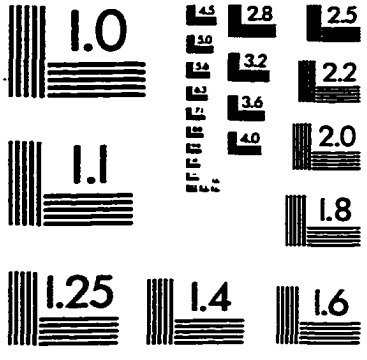
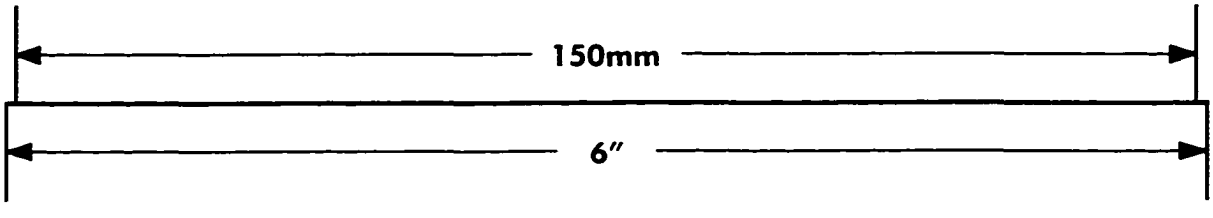
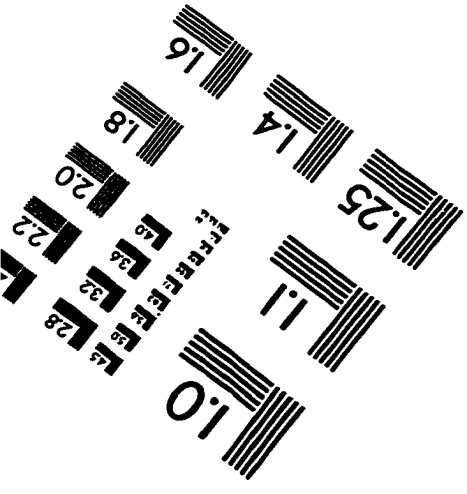
IMAGE EVALUATION TEST TARGET (QA-3)

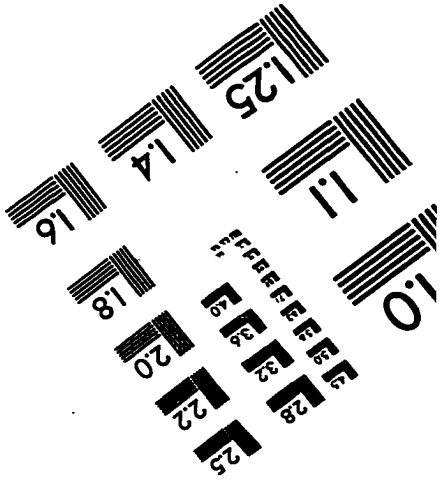












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