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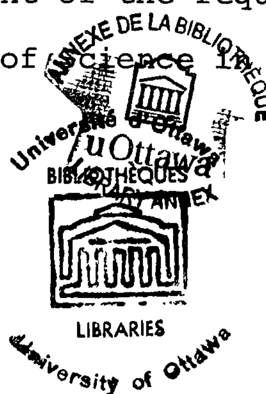
THE EFFECT OF A SPRINT TRAINING PROGRAM UPON FRUCTOSE 1,6-DIPHOSPHATASE ACTIVITY IN THE SOLEUS AND THE WHITE PORTION OF THE VASTUS LATERALIS OF THE RAT

by Marie Beauregard

Thesis presented to the School of Graduate Studies in partial fulfilment of the requirements for the degree of Master of Science in Kinanthropology



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CHAPTER 1

INTRODUCTION

Muscle cells receive all their energy through chemical reactions. All these reactions have to be enzyme-catalyzed in order to provide the energy at a rate compatible with life. Enzymes are subjected to many controls which link their functions to the needs and the demands of the cell.

Much research in exercise physiology is concerned with the effects of training and exercise on enzyme activity. Due to the highly specific nature of enzymes and their kinetics, such studies give us information about the mechanisms which underlie the specificity of adaptation to the various types of training.

Four reactions of the glycolytic pathway cannot simply be reversed. These irreversible glycolytic steps, which have to be bypassed in order to re-convert pyruvate and lactate into glucose, are catalyzed by pyruvate kinase, phosphoglycerate kinase, phosphofructose kinase (PFK) and hexokinase. The reversal of the reactions catalyzed by the above enzymes requires the antagonistic enzymes pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), fructose 1, 6-diphosphatase (FDPase) and glucose -6-phosphatase respectively. Dihydroxyacetone phosphate (DHAP)

and α-glycerol-phosphate are the most probable glycolytic intermediates that could be reconverted into glycogen through muscle gluconeogenesis (Krebs et al, 1965; Opie et al, 1967). FDPase, an enzyme located higher in this metabolic pathway is the subject of this present study.

The reasons for the existence of this enzyme in skeletal muscle are still unclear, nowever two concepts have been presented which offer possible explanations. One concept comes from studies concerned with the metabolism of liver and kidney and has established a gluconeogenic role for FDPase (Mokrasch et al, 1956; Exton et al, 1965; Friedman et al, 1965; Newsholme et al, 1966). However, other enzymes from this pathway have been found to be very low, if not absent, in skeletal muscle making it unlikely that significant amounts of gluconeogenesis occur in this tissue (Barnard et al, 1970; Newsholme et al, 1973; Bloxham 1974). Only indirect evidence supports the second concept that FDPase participates with PFK in the regulation of the glycolytic flux (Newsholme et al, 1973).

It was deemed important to investigate the effect of a sprint training program on the activity of FDPase since this type of exercise is known to produce the highest level of muscle lactate (Keul, 1973), blood lactate (Armstrong et al, 1974), and to predominantly cause the recruitment of fast twitch (FT) fibers which possess the highest FDPase activity (Table 1).

1. Statement of the Problem

The aim of this study was to investigate the effect of a sprint training program on FDPase activity in soleus and white vastus lateralis of the rat.

2. Definition of Terms

FRUCTOSE 1, 6-DIPHOSPHATASE: (E.C. 3.1.3.11) fructose 1, 6-diphosphate, 1-phosphohydrolase, a gluconeogenic enzyme responsible for the conversion of fructose 1, 6-diphosphate (F1,6-P) to fructose-6-phosphate (F-6-P).

SPRINT TRAINING: A type of high-intensity, short-duration training designed to force the recruitment of predominantly FT fiber, to produce relatively high muscle and blood lactate levels, and to stress the anaerobic metabolic system.

3. Abbreviations

AMP:	adenosine monophosphate
ATP:	adenosine triphosphate
ATPase:	adenosine triphosphatase
DHAP:	dihydroxyacetone phosphate
EDTA:	ethylenediamine tetracyclic acid
F-6-P:	fructose-6-phosphate
F1,6-P	fructose 1, 6-diphosphate
FDPase:	fructose 1, 6-diphosphatase
FOG:	fast oxidative glycolytic fiber
FT:	fast twitch fiber
K_M:	Michaelis constant
Mg^{++}:	magnesium ion
Mn^{++}:	manganese ion
NADP:	nicotinamide adenine diphosphate
O.D:	optical density
PEPCK:	phosphoenolpyruvate carboxykinase
PFK:	phosphofructose kinase
ST:	slow twitch fiber
TCA:	tricarboxylic acid cycle

CHAPTER 2

REVIEW OF RELATED LITERATURE

1. Muscle General Characteristics

Animal muscle fibers can be classified in three categories depending upon their relative contractile properties and enzymatic activities (Close, 1972), the FT possess high myofibrillar ATPase activity. The activity of their glycolytic enzymes is relatively high whereas their mitochondrial oxidative enzyme activities are relatively low. These fibers are easily fatiguable and thus are well suited for the high-intensity, short-duration type of work (Bass *et al*, 1973).

The slow twitch fibers (ST) have low myofibrillar ATPase activity; their capacity for mitochondrial oxidation is high but their glycolytic potential is low. They contract slowly, are fatigue-resistant fibers, and thus are well equipped for low-intensity, long-duration work (Bass *et al*, 1973).

In animal species it is possible to identify a third type of fiber which has high myofibrillar ATPase activity, high oxidative capacity and intermediate glycolytic enzyme activities. They are named fast-oxidative-glycolytic fiber (FOG). The characteristics of the above three fiber types

are summarized in Table 1.

Muscle varies in its fiber composition but it is generally accepted that each motor unit is composed of only one kind of fiber, thus implying that a specific recruitment pattern for a specific type of work is possible.

In mammalian species, the activity of FDPase is usually higher in white than in red muscles (Krebs et al, 1965; Opie et al, 1967; Bass et al, 1969; Newsholme et al, 1973).

TABLE 1

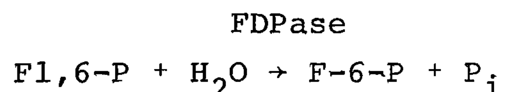
MAMMALIAN SKELETAL MUSCLE

	FT	ST	FOG
OXIDATIVE POTENTIAL	low	high	high
GLYCOLYTIC POTENTIAL	high	low	intermediate
CONTRACTION SPEED	fast	slow	fast
FDPase ACTIVITY*	relatively high	relatively low	unknown

* From Krebs et al, 1965; Opie et al, 1967; Bass et al, 1969.

2. Fructose 1, 6-Diphosphatase

FDPase is responsible for the conversion of fructose 1, 6-diphosphate (F1,6-P) to fructose-6-phosphate (F6P) as schematically described by the reaction below:



$$F1,6\text{-}P + H_2O \xrightarrow{\text{FDPase}} F\text{-}6\text{-}P + P_i$$

This reaction represents the reverse step of the PFK reaction in the glycolytic pathway. The FDPase reaction is one of those necessary for the gluconeogenic process, e.g., the formation of glucose from carbohydrate and non-carbohydrate precursors (Figure 1).

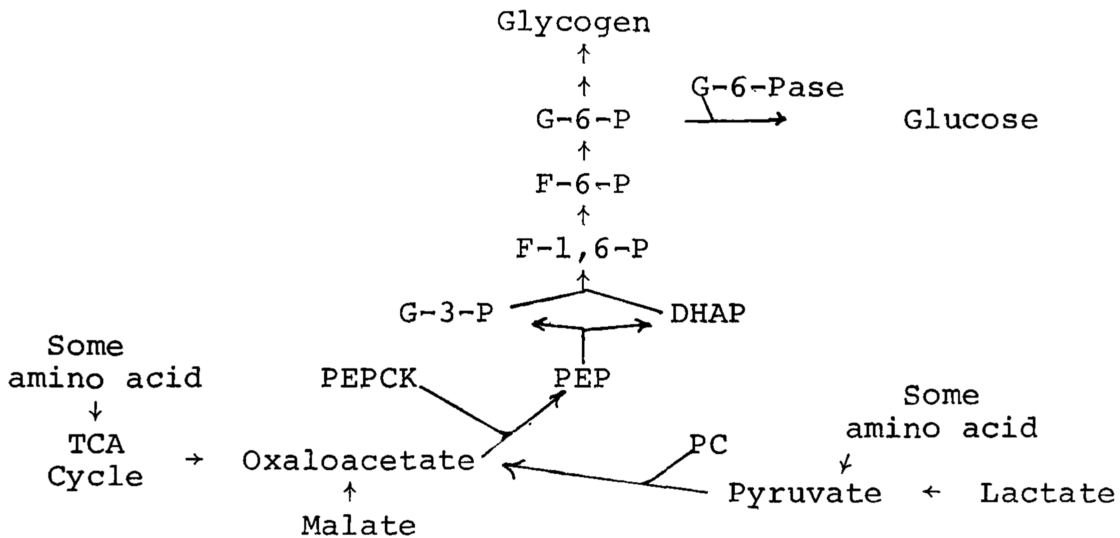


Figure 1:- Gluconeogenic Pathway (adapted from Lehninger, 1972).

It was not until 1943 that the existence of a specific phosphatase for the 1-phosphate group was confirmed from studies of mammalian liver and kidney extracts (Gomori, 1943). Gomori was not able to recover any enzymatic activity from skeletal muscle.

Hers in 1957 (as reported by Newsholme *et al*, 1962) showed that rabbit skeletal muscle incubated with F1,6-P liberated P_i. However he did not correlate this liberation of P_i with the formation of F-6-P.

Eight years later, Krebs and Woodford (1965) described the existence of FDPase activity in mammalian muscles. Their findings were later confirmed (Opie *et al*, 1967).

In addition to muscle, liver (Byrne *et al*, 1971; Cohen *et al*, 1971; Geller *et al*, 1971; 1972) and kidney (Krebs, 1964; Mendicino *et al*, 1963; Marcus *et al*, 1967; 1968; Nakashima *et al*, 1970), both of which are accepted as gluconeogenic tissues, possess FDPase activity in many species. FDPase activity has been reported to be negligible in rat heart, smooth muscle and some insect flight muscle (Newsholme *et al*, 1962; 1973; Krebs *et al*, 1965; Opie *et al*, 1967). Hence skeletal muscle FDPase was thought to play a gluconeogenic role.

3. Properties and Characteristics of Muscle FDPase

Although liver FDPase has been extensively studied by Pontremoli and Horecker (Horecker *et al*, 1970), the properties and characteristics of the muscle enzyme are of particular importance to the present study.

Muscle FDPase exhibits a pH optimum in the neutral range of 6.8 - 7.5 in studies of many species using crude extracts (Krebs *et al*, 1965) as well as in purified rabbit muscle extract (Fernando *et al*, 1968; 1969). The presence of chelator agents like ethylenediamine tetracyclic acid (EDTA) and mercaptoethanol as well as magnesium (Mg^{++}) and

manganese (Mn^{++}) ions affect this pH optimum. Although a more alkaline pH optimum has been reported (9.4 - 9.5) (Datta et al, 1973; Vantol et al, 1972) with highly purified enzyme assays, the previously mentioned neutral range has been verified for crude extract preparations.

The molecular weight of the rabbit enzyme has been estimated between 133,000 and 141,800 (Fernando et al, 1968; Black et al, 1972). Like the liver enzyme, muscle FDPase is formed of four subunits (Black et al, 1972).

The amino acid composition of the liver and muscle FDPase show that these two proteins are different in nature although they share some properties (Fernando et al, 1969; Black et al, 1972). The lack of identical nature of the muscle and liver FDPase is also illustrated by their difference in immunological properties and electrophoretic motility (Enser et al, 1969). The muscle enzyme has a very high affinity for its substrate; a Km value equal to 0.1 micro-mole was found in frog and rabbit purified extracts (Salas et al, 1964). High concentrations of F1,6-P in crude extract assays are known to inhibit the enzyme activity (Krebs et al, 1965). To be maximally active the enzyme required the presence of a chelating agent like EDTA (Krebs et al, 1965; Opie et al, 1967), histidine and citrate (Datta et al, 1974) and divalent cations like Mg^{++} and Mn^{++} (Sato et al, 1969; Fu et al, 1973; Fernando et al, 1968) or both (Datta et al, 1974; Vantol et al, 1972). These agents and cations appear to reduce the enzyme sensitivity to AMP

inhibition. This has been explained, at least in the case of Mg^{++}, by the formation of an ion-phosphate complex decreasing the concentration of free AMP (Fernando et al, 1970; Vantol et al, 1972; Fu et al, 1973). The action of Mn^{++} is still unclear but apparently much more complex than that of Mg^{++} (Fernando et al, 1970; Vantol et al, 1972).

AMP is known to be the most potent inhibitor of muscle FDPase. Concentrations between 2.0 micro-moles to 3.4 micro-moles of AMP produced 50% inhibition of the enzyme's activity (Krebs et al, 1965; Fernando et al, 1968; Black et al, 1972). It is assumed that three to four binding sites of AMP exist per tetramer (Fernando et al, 1968) making muscle FDPase an allosteric enzyme. This inhibition is highly specific to this phosphate compound (Salas et al, 1964) and is due to sulfhydryl groups present in the tetramer (Vantol et al, 1974).

Other reported activators of this muscle enzyme are creatine-phosphate, citrate, and inorganic phosphate (Fu et al, 1973) although some controversy still exists with this other group (Datta et al, 1974). Some activation is reported with ammonium chloride, potassium chloride, and cesium chloride (Black et al, 1972).

In summary these studies have shown that FDPase activity is controlled by many factors (e.g. AMP, Mg^{++}, Mn^{++} etc.), other than the substrate concentration which makes this enzyme one of the regulatory enzymes (Newsholme et al, 1973).

4. Theory of the Substrate Cycling

The fact that FDPase has a low Km value for its substrate, that its activity is very sensitive to the AMP content of the cell, that its maximal activity is only 2 to 10% that of PFK, led Newsholme *et al*, (1972) to reconsider the existing hypothesis regarding FDPase's gluconeogenic role in skeletal muscle.

Their theoretical model proposed that FDPase along with PFK may provide a sensitive control mechanism for the regulation of glycolysis. It was hypothesized that PFK is continually active in muscle even at rest. Since AMP content in resting muscle is low, FDPase is also active producing a futile substrate cycling during which ATP is hydrolyzed into ADP and P_i (Figure 2). Heat is produced during this cycle.

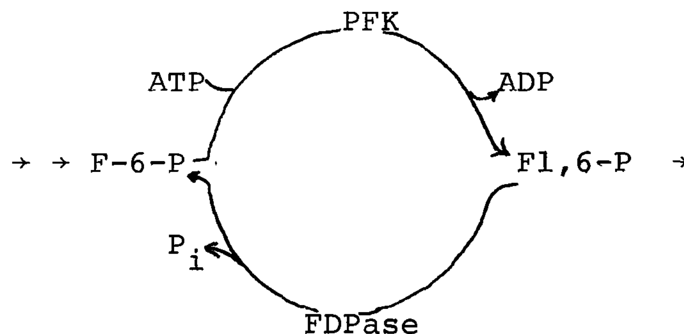


Figure 2:- Substrate Cycling Model (from Newsholme *et al*, 1973, p. 122).

Because PFK is an allosteric enzyme, and since it is always active, it would be possible to increase the rate at which glycolysis can be "switched on." This rate of activation can be markedly increased due to the fact that the metabolic flux through glycolysis is not dependent upon the activation of only one enzyme, (e.g. PFK), but rather to the activation of PFK and simultaneous inactivation of FDPase.

The second advantage of this cycle would be that the change in concentration of AMP could be kept within a certain range and still be responsible for the 440% increase of PFK activity which can happen during glycolysis (Newsholme et al, 1972). This maximal increase in PFK activity would normally require large changes in AMP concentration. However the adenylate kinase reaction preset the range over which the AMP concentration changed in the cell and this could not be increased. (Newsholme et al, 1972.) Therefore, an amplification mechanism is needed. Since it is the PFK/FDPase ratio that regulates the glycolytic flux, and that PFK is activated by AMP while FDPase is simultaneously inhibited by this phosphate compound, it can be seen that Newsholme's substrate cycling concept provides the rationale for an amplification system by which AMP concentration can regulate the glycolytic pathway.

This theoretical model is strengthened by the fact that FDPase is not detectable in muscles that are tonically active such as heart and smooth muscle (Newsholme et al, (1972). In these muscles PFK activity would not have to be

modulated over as great a range as that in skeletal muscle. It is also interesting to note that FDPase activity is higher in glycolytic fiber (e.g. FT) than in ST of the skeletal muscles of many mammalian species (Krebs *et al*, 1965; Opie *et al*, 1967; Bass *et al*, 1969); although the higher FDPase activity in FT seems to indicate that this tissue is potentially gluconeogenic, these fibers possess higher PFK activity than the ST fiber (Saubert IV *et al*, 1973) thus implying that FDPase could have a regulatory role as well as a gluconeogenic role.

5. The Effect of Exercise on Muscle Gluconeogenesis and FDPase Activity

In 1968, Barnard reported that Meyerhof, in 1920-1921, estimated that in isolated frog muscle 80% of the lactate was converted back to glycogen, the other part being oxidized to CO_2 and water. This work has never been confirmed. In fact, in 1935 Sacks *et al*, felt that in intact animals and *in situ* muscle preparations most of the lactate diffused into the blood and that none was transformed into glycogen in the muscle. Janssen *et al*, in 1925 as reported by Cori (1931) also showed that sodium lactate infusion in hind legs did not produce an increase in muscle glycogen. In 1952 Villee *et al*, found that glucose-6-phosphatase was absent in rat diaphragm. However they found that injection of labeled c^{14}-pyruvate increased the glycogen synthesis. The fact that rabbit skeletal muscle was devoid of pyruvate

carboxylase strongly suggested that gluconeogenesis was not occuring at a significant rate in this tissue (Keech _et al_, 1963). However Opie and Newsholme (1967) found that PEPCK was in fact present in FT fiber. Since these fibers also possess FDPase activity, it was stated that gluconeogenesis could occur in muscle even if pyruvate carboxylase was not present. They hypothesized that accumulation of glycolytic intermediates, (e.g. αGP & DHAP) might possibly be used to form glycogen in the muscle (Figure 1).

Apparently only one study in the literature is directly related to the effect of exercise on FDPase activity in muscle (Krebs _et al_, 1963). Eleven groups of male Wistar rats were forced to swim for varying times at 37^{0}C. The average period of exercise was five hours per day for eleven experimental periods varying from six to ten days each. No significant change in activity was reported in any exercise period. The negative results reported in this study could be due to the fact that this type of endurance work did not require the recruitment of the FT fiber. The effect of an anaerobic, sprint kind of training on FDPase activity is therefore still unknown.

6. Sprint Training and Exercise

Relatively few researchers in exercise physiology have been preoccupied with the effect of a sprint training program on the organism.

Glycogen depletion studies on vastus lateralis from humans pedalling for six one-minute sprints, with ten minutes rest between each sprint, which ellicited an energy expenditure of 150% MVO_2, showed that FT fibers are the first to become depleted of their glycogen (Gollnick et al, 1973). In rats at an estimated level of work intensity of 162% MVO_2 FT fibers of the plantaris are the most depleted of their glycogen (Armstrong et al, 1974). Since FT possess higher phosphrylase activity (Bass et al, 1969) than ST, it is difficult to state that ST fiber are or are not recruited to the same extent at this level of intensity since they would lose their glycogen more slowly. But these studies definitively show that as the level of work intensity increases, more and more FT are recruited.

Sprint training ellicits different enzymatic changes in different muscles, depending upon their fiber composition. A twenty-one day training period which required rats to run for forty-five seconds four times a day at 80 m/min. against 30^0 incline produced some adaptations (Staudte et al, 1973). Increases in hexokinase, citrate synthetase, succinate dehydrogenase were reported in the rectus femoris and soleus. A more pronounced increase in glycogen phosphorylase and triose-phosphate dehydrogenase in soleus as compared to rectus femoris was shown. However these two enzymes are respectively 15 times and 3 times higher in rectus femoris than in soleus. They also indicated that the soleus became faster decreasing its tetanic contraction times by 15% and

maximal tetanic tension increased by 18%. Similar but non-significant, changes were reported for the rectus femoris.

Saubert IV et al, (1973) imposed a sprint training regimen of eleven weeks in a motor driven wheel on rats. They were allowed to rest thirty seconds and forced to work for the same period of time. They started their training by five sprints at 16.1 m/min. until they could run eighteen sprints of 80 m/min. During the final week of training, they reported significant increases only in phosphorylase and pyruvate kinase in the soleus. White and red portions of the gastrocnemius and red vastus lateralis were also studied. The non-increase in SDH activity was used in this study to indicate that the training program did not stress the aerobic capacity of the body, the program being anaerobic in nature.

Training identical to the above did not produce any changes in the activity of myosin-ATPase in the red and white portion of the gastrocnemius (Bagby et al, 1972).

Storage of phosphate compounds was also affected by sprint training. Training programs in which rats run six to eight intervals and in which the rats were able to run at 81.1 m/min. for the last three weeks of training, increased creatine phosphate in the gastroc-plantaris as compared to the endurance group which was subjected to thirty minutes run at 34 m/min. during the same period of training. However, no significant difference existed between control

values and sprint training (Gale *et al*, 1971).

Literature on the effect of sprint training programs upon the contractile properties of skeletal muscles is conflicting since any changes in the contractile characteristics of the muscle should be reflected in the activity of myosin-ATPase. Therefore sprint training studies have shown to be effective in changing mostly glycolytic enzyme activities, especially in the ST fibers.

However the effect of sprint training on enzyme activities related to gluconeogenesis, such as FDPase in FT fibers, is unknown.

7. Summary of the Related Literature

The present review of literature can be summarized as follows. The physiologic role of muscle FDPase is still unclear but this enzyme is under many controls which make it a regulatory enzyme. The long duration type of exercise did not affect its activity (Krebs *et al*, 1963). Gluconeogenesis can possibly occur in skeletal muscle but whether or not this pathway is significantly used in this tissue remains to be determined. FT fiber appears to be the most probable site of gluconeogenesis in skeletal muscle since, in these fibers, FDPase activity has been reported in significant amounts. Sprint training has produced some enzymatic adaptations; however, its effect on this enzyme remains to be investigated.

CHAPTER 3

METHODOLOGY

1. Description of the Subjects

A total of 29 rats were obtained from Bio Breeding Laboratories of Canada in Ottawa. They were male Sprague-Dawley rats weighing approximately 235 grams and were 49 days old. Upon their arrival, the animals were weighed and housed in separate cages. Food and water was provided *ad libitum* throughout the experiment. They were immediately subjected to an artificial 12 hour day-night cycle (light 1 a.m. to 1 p.m.). The first day they were individually introduced to the treadmill for two minutes. On the second, third, and fifth days the animals were tested for their ability to run. Each rat was allowed two trials to attempt a run of at least 30 seconds at a speed of 22 m/min. without hesitation. After the above five-day acclimatization period, the animals were divided into three groups. One group, consisting of the 17 animals who best responded to the acclimatization procedure, were assigned to the training group (TRAIN) to undergo 11 weeks of sprint training, as described below. Another group (CON) of 6 animals was sacrificed as a pre-training control group (54 days old) and the remaining 6 animals were sacrificed at the same time as

the trained group to serve as post-training controls for the age effect. At the end of the training period, the six animals who best met the requirements of their training program (Appendix A) were selected for sacrifice. Sacrifice occured two days after the final training session when the animals in both the AGE and TRAIN group were 129 days old. The experimental design is outlined in Table 2.

TABLE 2

EXPERIMENTAL DESIGN

	TIME (days)									
Group	0	1	2	3	4	5	→	127	128	129
CON	In	A	A	A	R	AX				
AGE	In	A	A	A	R	A	sedentary→	R	R	X
TRAIN	In	A	A	A	R	A	sprint train→	R	R	X

Key:
In = Animal into the laboratory
A = Acclimatization
R = Rest
X = Sacrifice

All the animals were handled daily and were weighed every second day with the weighing always occurring at the same time.

2. Training Program

The first four weeks of the program were planned to gradually bring the rats to a treadmill[1] running performance level of two bouts daily (at 2:30 and 7:30 p.m.) of eight sprints of 15 seconds duration at 80m/min. up a 30^0 incline, each followed by 20 seconds rest (see Appendix A). The protocol used for this work was similar to that of a previous study in which a value of 123mg. %/% in blood lactate was produced (Reardon, 1975). The training was done five consecutive days of each week. A 60 second warm-up followed by 60 seconds rest preceded each training session. During this warm-up period, the speed of the treadmill was progressively increased until the speed planned for that session was reached. This warm-up and rest period was extended to 2 minutes when the running speed was 60m/min. or higher. During each run, 60 to 70 volts were maintained across the shock grid at the rear of the running compartment. During the last four weeks the animals were required to maintain the same level of performance since the 80m/min. speed was the maximum the animals could attain.

3. Sacrifice Procedure

The animals were killed by decapitation. Following the sacrifice, the soleus and white superficial portion of the vastus lateralis of both hind legs were removed and the

---

[1]Quinton Model 42-15, motor driven with individual compartments.

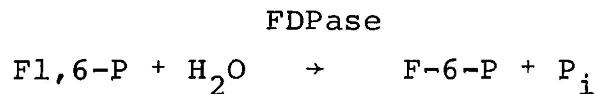
muscle preparation was immediately placed in a beaker on ice.

4. Tissue Analysis

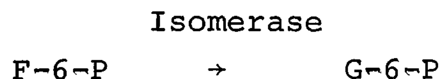
The muscles were washed with a Ringer solution (Appendix B), and then rapidly weighed. The tissue was finely cut with scissors and homogenized for two minutes in eight volumes of distilled water containing 1 mM of EDTA at pH of 7.3. The extract was centrifuged for 40 minutes at 20,000 g. The supernatant was used for the assay and for the protein estimation.

5. Spectrophotometric Measurements

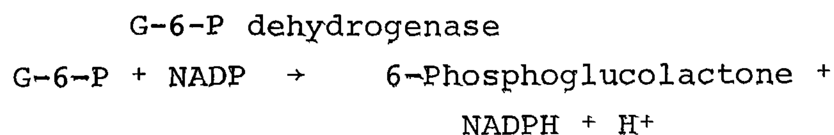
FDPase activity was measured on a Gilford spectrophotometer Model 2400-Z with a 0.1 O.D (optical density) range on the chart paper. The rate of reduction of NADP was followed at a wavelength of 340nm. The principle behind the procedure was the coupling of the FDPase reaction with the glucose-6-phosphate dehydrogenase and the phospho-glucoisomerase reactions as indicated below:



$$\text{F1,6-P} + H_2O \xrightarrow{\text{FDPase}} \text{F-6-P} + P_i$$



$$\text{F-6-P} \xrightarrow{\text{Isomerase}} \text{G-6-P}$$



$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-P dehydrogenase}} \text{6-Phosphoglucolactone} + \text{NADPH} + H^+$$

NADPH absorbs light at a wavelength of 340 nm while NADP does not. Since one mole of G-6-P is formed from each mole of F-6-P produced and G-6-P subsequently disappears when each mole of NADPH is formed, it is possible to measure the reduction of NADP as an indicator of the activity of FDPase. All determinations were made in duplicate. The substrate was omitted from the blank. The extinction coefficient used for NADPH was 6.22 mM^{-1} cm^{-1} (Bergmeryer, 1963). The enzyme activity was expressed in terms of micro-moles of F1,6-P hydrolyzed per minute, per g. of wet weight of muscle, and mg. of protein.

FDPase was assayed at 28^0 C and at pH 7.5 in a medium containing the following:

	Compound	Final Concentration
a)	Tris-HCl buffer, pH 7.5	40mM
b)	$MnCl_2$	0.2mM
c)	F1,6-P	0.2mM
d)	NADP	0.2mM
e)	enzyme preparation	50ug - 150ug
f)	phosphoglucoisomerase	2ug
g)	glucose-6-dehydrogenase	2ug
h)	ATP	0.05mM
i)	phosphoenol pyruvate	0.5mM
j)	myokinase	5ug
k)	pyruvate kinase	10ug
l)	EDTA	0.1mM

The compounds h, i, j, k form an AMP trapping system (Black _et al_, 1972). The total volume of the assay was one milliliter and the reaction was started by adding F1,6-P to the medium after a pre-incubation of five minutes with the trapping system.

6. Protein Estimation

Protein estimation was made by a modified Lowry procedure (Appendix C). Twenty micro-liters of homogenate were used for protein estimation: the measurements were made in triplicate.

7. Analysis of the Data

The statistical analysis of FDPase was done by a two-way analysis of variance. The independent variables were muscle with the subcategories: soleus and white vastus and treatment with the three subcategories AGE, TRAIN and CON. The dependent variables were enzyme activity and protein estimation. Where significance ($p \geq .05$) was found, the Scheffe test was used to identify the specific contrasts between groups which caused the significance.

CHAPTER 4

RESULTS AND DISCUSSION

1. Results

The purpose of this study was to investigate the effect of a sprint training program on the activity of FDPase in rat soleus and white vastus lateralis. The results will be analyzed as follows: a) the training aspect of the experiment, which includes training performance and body weight data, b) the activity of FDPase in both muscles, expressed in terms of muscle wet weight, c) the specific activity of FDPase in soleus and white vastus, expressed in terms of mg. of protein, and, d) protein estimation.

a) Training aspect

The training program was of 11 weeks duration at the end of which the rats were able to run 8 repeated runs twice daily at 80m/min. up a 30^{0} incline for 15 seconds with 20 seconds rest between each bout (Appendix A). The first 7 weeks were used to bring the rat to their maximal work performance. After this period, the workload was kept constant.

The graph representing the growth of the rats as related by body weight in TRAIN and AGE group is in Appendix D. It can be seen that the TRAIN group did not gain as much weight as the AGE group.

b) Activity of FDPase in the tissue

The data from each muscle in terms of FDPase activity related to the wet weight of the muscle are in Appendix F. Tables 3 and 6 represent the statistical treatment of these data where the enzyme activity is expressed as umoles of F1,6-P hydrolyzed/min./g. of tissue.

TABLE 3

ANOVA TREATMENT UPON THE ENZYME ACTIVITY (umole/min/g)

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio
Treatment	2	0.025	0.012	2.52
Muscle	1	2.136	2.136	434.20 *
Interaction	2	0.025	0.012	2.52
Error	30	0.148	0.005	

* Significant at the level of $p \leq 0.01$

There is no statistically significant difference between TRAIN, AGE, and CON. However, there is a significantly

greater activity in the white vastus than in the soleus.

c) Specific activity

The individual data representing the enzyme activity expressed in umoles of F1,6-P hydrolyzed /min./mg. of protein are in Appendix G. The ANOVA table of these data are presented in Tables 4 and 6.

TABLE 4

ANOVA TREATMENT UPON THE SPECIFIC ENZYME ACTIVITY (umole/min/mg)

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio
Treatment	2	$.003\times10^{-2}$	0.001×10^{-2}	2.07
Muscle	1	.001	0.001	204.79 *
Interaction	2	$.003\times10^{-2}$	0.001×10^{-2}	2.08
Error	30	$.002\times10^{-1}$	0.007×10^{-3}	

* Significant at the level of $p \leq 0.01$

When the enzyme activity is related to the protein content of the homogenate, there is no statistical difference between TRAIN, AGE and CON. However the specific activity of the soleus is significantly lower than the white vastus lateralis.

d) Protein estimation

Data in Appendix H represent the mean value for each different homogenate studied. Tables 5 and 6 show the statistical analysis of the data.

TABLE 5

ANOVA TREATMENT UPON PROTEIN ESTIMATION DATA (for 20 ul.)

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio
Treatment	2	9346.510	4673.255	11.67 *
Muscle	1	983.031	983.031	2.45
Interaction	2	1926.753	963.376	2.40
Error	30	12017.449	400.582	

* Significant at $p \leq 0.01$

There is a statistically significant difference between TRAIN, AGE and CON groups. To find which contrasts were significant, a Scheffe test was done. There was a significant difference between CON and both the AGE and TRAIN groups, for the vastus lateralis ($p \leq 0.05$). The homogenate of the AGE group showed the highest value for protein estimation while the CON was the lowest. No statistically significant difference was found in the soleus although the data showed a trend in the same direction as those of the white vastus.

TABLE 6

MEAN ENZYME ACTIVITIES AND PROTEIN ESTIMATIONS

		Soleus Mean ± S.D.	White Vastus Mean ± S.D.
CONTROL	Enzyme Activity (um/min./g.)	$.188 \times 10^{-1} \pm .013$	$.433 \pm .081$
	Enzyme Activity (um/min./mg.)	$.005 \times 10^{-1} \pm .0001$	$.155 \times 10^{-1} \pm .004$
	Protein Estimation (ug)	78.0 ± 15.9	68.0 ± 12.1
AGE	Enzyme Activity (um/min./g.)	$.156 \times 10^{-1} \pm .005$	$.526 \pm .099$
	Enzyme Activity (um/min./mg.)	$.004 \times 10^{-1} \pm .0001$	$.144 \times 10^{-1} \pm .003$
	Protein Estimation (ug)	101.9 ± 14.3	119.5 ± 23.4
TRAIN	Enzyme Activity (um/min./g.)	$.201 \times 10^{-1} \pm .007$	$.557 \pm .089$
	Enzyme Activity (um/min./mg.)	$.006 \times 10^{-1} \pm .0001$	$.123 \times 10^{-1} \pm .003$
	Protein Estimation (ug)	90.2 ± 23.9	113.8 ± 16.9

2. Discussion

a) Training aspect

The training program did bring the rats to the target sprint type of performance which consisted of eight repeated runs at 80 m/min. for 15 seconds each, with 20 seconds rest between each run. The 30^0 incline further increased the anaerobic stress upon the rats' performance. At the end of the training program, it was difficult for the rats to sustain this high workload. This could be explained partially by their high body weight at this time (Appendix D). The effective length of the training program as well as their running performance seems to be limited by their body weight. The rats did show some signs of over-training (fatigue and refusal to run) during the period of the 16th day to the 23rd day (Appendix A). During this period the speed of the treadmill was kept constant and the number of repetitions was increased slowly until they were able to run at the fastest speed.

b) FDPase activity

When the enzymatic activity of FDPase was expressed in terms of wet weight of muscle, values between 0.557 and 0.433 umoles F1,6-P hydrolyzed/min./g. of muscle for the white vastus and between 0.0201 and 0.0156 for the soleus were obtained. These data are in accord with previous work since rat quadriceps FDPase activity has been reported as 0.3 umoles F1,6-P hydrolyzed/min/g. of tissue at 25^0 C

(Crabtree *et al*, 1972) and rabbit soleus FDPase activity at the same temperature was 0.058 (Bass *et al*, 1969). The results of this study confirmed the higher FDPase activity in fast than slow muscle as previously reported (Krebs *et al*, 1965; Opie *et al*, 1967; Bass *et al*, 1969). When the enzyme activities were expressed in terms of mg. of protein, this difference between muscle types was still present.

c) Age and training effect

The training did not produce any significant differences in FDPase activity. Both AGE and TRAIN groups had higher protein estimations when compared with the CON group for the white vastus. It thus appears that the aging effect is responsible for this increase of protein content in the 20 ul aliquots of the muscle homogenates. The fact that only the white vastus homogenates increased significantly in protein content with AGE could be due to the specific localization of this muscle (e.g. superficial muscle) as well as its predominently FT fiber population since sprint training of the type used in this study causes the recruitment of FT fibers.

d) Significance of the results

The sprint training exercises are known to increase levels of resting muscle glycogen and to produce high concentrations of blood lactate (up to 20mM) (Armstrong *et al*, 1974). If gluconeogenesis does occur in exercised skeletal muscle, it would be feasible that the repeated

stimulus of a sprint training program would produce an increase in the activity of the gluconeogenic enzymes. However the data in the present study, do not support this hypothesis.

The "non-increase" in FDPase activity is particularly interesting in light of reports that sprint training does not affect PFK activity in soleus, white and red gastrocnemius and red vastus (Saubert IV et al, 1973). These findings along with those of the present study better support the concept that the major importance of FDPase existence in muscle is not related to its gluconeogenic role but rather to regulation, with PFK, of the metabolic flux through glycolysis, as proposed by Newsholme et al, (1973). It has been recently hypothesized that the rapid muscle lactate disappearance during recovery from three series of 1 minute maximal performances on the bicycle ergometer was responsible for the observed high rate of post-exercise glycogen resynthesis in muscle (Hermansen et al, 1976). The present study does not support the hypothesis that lactate produced during short-duration, high-intensity exercise has a gluconeogenic fate within skeletal muscle. However enzyme activity studies such as this one should be interpreted with caution as this has been well reviewed by Moussa et al, (1975). The major limitation of these studies is the fact that the optimum conditions of the medium in which the biochemical analysis takes place does not necessarily reflect the existing conditions in the in vivo cell and that the

degree to which the cell integrity is preserved is not known.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

1. Conclusion

The purpose of this study was to investigate the effect of a sprint training program upon FDPase activity in soleus and white vastus lateralis of the rat. From the results of the present study, it was concluded that FDPase activity is not significantly changed by this type of training and that therefore FDPase may be more involved in the regulation of glycolysis than in the regulation of gluconeogenesis in skeletal muscle.

2. Recommendations

In view of the decrease in the training performance of the rat at the end of the training program, it is recommended that the rat should be killed while reaching its maximal performance.

The present study was restricted to an investigation of the effect of short-duration, high-intensity work upon only one gluconeogenic enzyme; further studies on the effect of a sprint training program upon other gluconeogenic enzymes are recommended.

APPENDIX A

SPRINT TRAINING PROGRAM

TABLE 7

A SPRINT TRAINING PROGRAM FOR RATS
BY EACH TRAINING DAY
(after Reardon 1975)

Day of Training	Grade	Speed	Work Interval	Rest Interval	Repetitions
	%	(m/min.)	(sec.)	(sec.)	
1	30	20	60	60	3
	30	25	60	60	3
2	30	30	45	45	3
	30	30	45	45	4
3	30	35	30	30	5
	30	35	30	30	5
4	30	35	30	30	8
	30	40	30	30	5
5	30	45	20	20	5
8	30	40	30	30	5
	30	45	20	20	5
9	30	45	20	20	8
	30	50	20	20	5
10	30	50	20	20	8
	30	55	20	20	5

Day of Training	Grade	Speed	Work Interval	Rest Interval	Repetitions
	%	(m/min.)	(sec.)	(sec.)	
11	30	55	15	20	8
	30	55	15	20	10
12	30	63	15	20	5
15	30	55	15	20	8
	30	55	15	20	10
16	30	63	15	20	6
	30	63	15	20	8
17	30	63	15	20	8
	30	63	15	20	10
18	30	63	15	20	10
	30	63	15	20	10
19	30	63	15	20	10
22	30	63	15	20	8
	30	63	15	20	8
23	30	63	15	20	10
24	30	65	15	20	8
	30	65	15	20	7
25	30	65	15	20	10
	30	65	15	20	10

Day of Training	Grade	Speed	Work Interval	Rest Interval	Repetitions
	%	(m/min.)	(sec.)	(sec.)	
26	30	65	15	20	10
29	30	63	15	20	12
	30	65	15	20	7
30	30	65	15	20	8
	30	65	15	20	12
31	30	65	15	20	8
	30	65	15	20	8
32	30	65	15	20	9
	30	65	15	20	9
33	30	65	15	20	10
36	30	65	15	20	9
	30	70	15	20	7
37	30	70	15	20	7
	30	70	15	20	7
38	30	70	15	20	8
	30	70	15	20	8
39	30	70	15	20	10
	30	70	15	20	11
40	30	73	15	20	9

Day of Training	Grade	Speed	Work Interval	Rest Interval	Repetitions
	%	(m/min.)	(sec.)	(sec.)	
43	30	75	15	20	7
	30	75	15	20	8
44	30	75	15	20	9
	30	75	15	20	9
45	30	75	15	20	8
	30	75	15	20	8
46	30	75	15	20	8
	30	75	15	20	10
47	30	75	15	20	10
50	30	75	15	20	9
	30	75	15	20	9
51	30	75	15	20	10
	30	80	15	20	8
52	30	80	15	20	8
	30	80	15	20	8
53	30	80	15	20	8
	30	80	15	20	8
54	30	80	15	20	8
	30	80	15	20	8

Day of Training	Grade	Speed	Work Interval	Rest Interval	Repetitions
	%	(m/min.)	(sec.)	(sec.)	
57	30	80	15	20	7
	30	80	15	20	7
58	30	80	15	20	7
	30	80	15	20	7
59	30	80	15	20	8
	30	80	15	20	8
60	-	-	-	-	-
61	30	80	15	20	10
64	30	80	15	20	8
	30	80	15	20	8
65	30	80	15	20	8
	30	80	15	20	8
66	30	80	15	20	8
	30	80	15	20	8
67	30	80	15	20	8
	30	80	15	20	8
68	30	80	15	20	8
71	30	80	15	20	8
	30	80	15	20	8

Day of Training	Grade	Speed	Work Interval	Rest Interval	Repetitions
	%	(m/min.)	(sec.)	(sec.)	
72	30	80	15	20	8
	30	80	15	20	8
73	30	80	15	20	8
	30	80	15	20	8
74	30	80	15	20	8
	30	80	15	20	8

APPENDIX B

COMPOSITION OF RINGER'S WASHING SOLUTION

RINGER SOLUTION

(Horowicz *et al*, 1970).

NaCl	115mM
KCl	2.3mM
$CaCl_2$	1.8mM
$NaHPO_4$	2.15mM
$Na_2H_2PO_4$	0.85mM

Figure 3:- Ringer solution.

APPENDIX C

PROTEIN ESTIMATION METHODS

MODIFIED LOWRY PROCEDURE FOR PROTEIN ESTIMATION

(Schacterle *et al*, (1973).)

REAGENTS

1. Alkaline copper reagent — 500 ml

10% Na_2CO_3	in water	50g
0.1% potassium tartrate		0.5g
0.05% $CuSO_4.5H_2O$		0.25g

(It is best to dissolve the copper sulphate first before adding the other compounds.)

2. Phenol reagent

   Dilute the 2N solution (purchased) 1:16 just before use (water)
   (1 → 16; 2 →32; 3 →48; 4 →64; 5 →80; 6 →96; 7 →112; 8 →128; 9 →144; 10 →160)

3. Albumin standard

   10 mg BSA/ml in water

   Working standard: dilute the stock standard 0.3 ml to 10 ml with 0.5 N NaOH
   (300 ug protein/ml)

4. 0.5 N NaOH

PROCEDURE

1. Prepare samples in 0.5 N NaOH, make up to 1 ml.
2. Prepare series of standards, also 1 ml.
3. Add 1 ml alkaline copper reagent to each tube. Mix. Leave at room temperature for 10 minutes.
4. Add 4 ml phenol reagent forcibly and rapidly. Mix.

5. Place the tubes in a water bath at 55º for 5 minutes.
6. Cool the tubes rapidly by placing them in ice or water.
7. Read absorbance at 650 nm.

Figure 4:- Modified Lowry procedure for protein estimation (Schacterle *et al*, (1973).)

APPENDIX D

BODY WEIGHT DATA

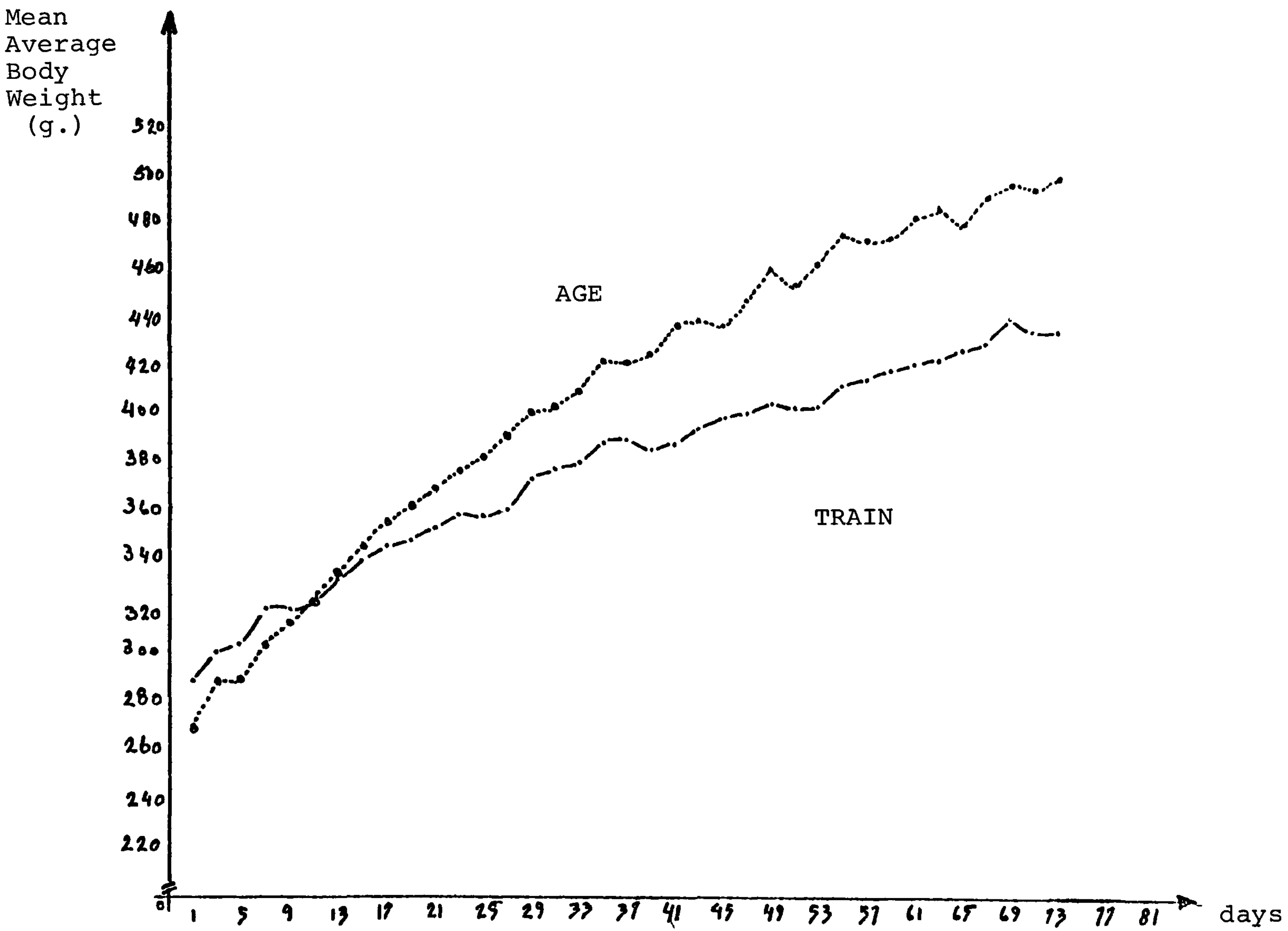


Figure 5:- Mean Body Weight of TRAIN and AGE Animals.

APPENDIX E

MUSCLE WEIGHTS

TABLE 8

MUSCLE WET WEIGHT* (g.)

Treatment	Muscle: Soleus	Muscle: White Vastus
	(g.)	(g.)
TRAIN	.310	.612
	.332	.925
	.258	.812
	.245	.816
	.295	.692
	.251	.520
Mean	.238	.729
AGE	.296	.900
	.275	.892
	.320	.890
	.269	.957
	.237	.875
	.275	.850
Mean	.279	.894
CONTROL	.160	.557
	.183	.364
	.182	.390
	.141	.567
	.145	.600
	.165	.660
Mean	.163	.523

* This weight represents the weight of both hind legs.

APPENDIX F

INDIVIDUAL VALUES FOR FDPase ACTIVITY PER G. OF MUSCLE

TABLE 9

FRUCTOSE 1,6-DIPHOSPHATASE ACTIVITY
(umoles FDP HYDROLYZED / MIN /G.)*

Treatment	Muscle: Soleus	Muscle: White Vastus
TRAIN	$.157 \times 10^{-1}$	.559
	$.134 \times 10^{-1}$	.627
	$.144 \times 10^{-1}$	.577
	$.352 \times 10^{-1}$	.679
	$.211 \times 10^{-1}$	.402
	$.207 \times 10^{-1}$	.498
Mean ± SD	$.201 \times 10^{-1} \pm .007$	.557 ± .089
AGE	$.103 \times 10^{-1}$	.499
	$.112 \times 10^{-1}$	.520
	$.227 \times 10^{-1}$	.489
	$.114 \times 10^{-1}$	.627
	$.197 \times 10^{-1}$	.662
	$.183 \times 10^{-1}$	.357
Mean ± SD	$.156 \times 10^{-1} \pm .005$	.526 ± .099
CONTROL	$.117 \times 10^{-1}$	.555
	$.468 \times 10^{-1}$	.514
	$.098 \times 10^{-1}$	.418
	$.023 \times 10^{-1}$	.434
	$.097 \times 10^{-1}$	.336
	$.012 \times 10^{-1}$	.344
Mean ± SD	$.188 \times 10^{-1} \pm .013$	.433 ± .081

* Values are averages for duplicate assays.

APPENDIX G

INDIVIDUAL VALUES FOR FDPase SPECIFIC ACTIVITY PER MG. OF PROTEIN

TABLE 10

FRUCTOSE 1,6-DIPHOSPHATASE ACTIVITY
(umoles of FDP HYDROLYZED
/MIN /MG OF PROTEIN)*

Treatment	Muscle: Soleus	Muscle: White Vastus
TRAIN	$.004x10^{-1}$	$.123x10^{-1}$
	$.0080x10^{-1}$	$.142x10^{-1}$
	$.005x10^{-1}$	$.128x10^{-1}$
	$.009x10^{-1}$	$.151x10^{-1}$
	$.007x10^{-1}$	$.065x10^{-1}$
	$.006x10^{-1}$	$.131x10^{-1}$
Mean ± SD	$.006x10^{-1}$ ± .0001	$.123x10^{-1}$ ± .003
AGE	$.002x10^{-1}$	$.129x10^{-1}$
	$.003x10^{-1}$	$.134x10^{-1}$
	$.005x10^{-1}$	$.085x10^{-1}$
	$.003x10^{-1}$	$.104x10^{-1}$
	$.005x10^{-1}$	$.155x10^{-1}$
	$.003x10^{-1}$	$.075x10^{-1}$
Mean ± SD	$.004x10^{-1}$ ± .0001	$.114x10^{-1}$ ± .003
CONTROL	$.004x10^{-1}$	$.213x10^{-1}$
	$.006x10^{-1}$	$.220x10^{-1}$
	$.004x10^{-1}$	$.135x10^{-1}$
	$.006x10^{-1}$	$.118x10^{-1}$
	$.002x10^{-1}$	$.131x10^{-1}$
	$.005x10^{-1}$	$.116x10^{-1}$
Mean ± SD	$.005x10^{-1}$ ± .0001	$.155x10^{-1}$ ± .004

* Values are averages for duplicate assays.

APPENDIX H

INDIVIDUAL VALUES FOR PROTEIN ESTIMATION

TABLE 11

PROTEIN ESTIMATION IN 20ul OF HOMOGENATE*
(ug)

Treatment	Muscle Soleus	White Vastus
TRAIN	95.7	115.5
	137.7	108.7
	63.0	109.3
	91.7	112.0
	73.2	146.8
	79.8	90.8
Mean ± SD	90.2 ± 23.9	113.8 ± 16.9
AGE	115.0	97.0
	84.0	96.7
	101.0	150.3
	100.3	151.3
	86.7	102.6
	124.3	119.0
Mean ± SD	101.9 ± 14.3	119.5 ± 23.4
CONTROL	65.7	65.3
	95.3	58.4
	57.7	54.7
	90.9	91.5
	94.8	64.0
	63.5	73.3
Mean ± SD	78.0 ± 15.9	68.0 ± 12.1

* Average values for triplicate assays.

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ABSTRACT

The effect of eleven weeks of a sprint training program on the activity of FDPase in rat soleus and white vastus lateralis was investigated.

It was found that the activity of FDPase did not change significantly following the training program whether the FDPase activity was expressed in umoles of substrate hydrolyzed /min./g. of tissue or umoles of substrate hydrolyzed /min./mg. of protein. Protein estimation was significantly affected by age but not training in the white vastus lateralis.

It was concluded that sprint training does not increase skeletal muscle's gluconeogenic potential, as indicated by FDPase activity, and that therefore FDPase may play a more regulatory than a gluconeogenic role in skeletal muscle.