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COMPARISON OF AEROBIC AND ANAEROBIC TRAINING ON GLYCOGEN DEPLETION AND ENZYMATIC ACTIVITY IN SKELETAL MUSCLE

by

© REAL J. GABORIAULT

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled: "Comparison of Aerobic and Anaerobic Training on Glycogen Depletion and Enzymatic Activity in Skeletal Muscle", submitted by Réal J. Gaboriault in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physical Education.

supervisor

External Examiner

Date 15/8/77

DEDICATION

A Louise, mon épouse, qui a contribué

sans relâche à l'obtention de mon diplôme

ABSTRACT

Sixty-two male Wistar rats were divided into five groups: sedentary control (CON), exercised aerobic (EAE), exercised anaerobic (EAN), trained aerobic (TAE), and trained anaerobic (TAN). Following a two-week progressive pre-training session, the aerobic animals (EAE and TAE and the anaerobic animals (EAN and TAN) were able to run continuously for 5 min at 40 m/min-15% incline, and intermittently for 10 bouts (15 sec work and 30 sec rest) 80 m/min-15% incline respectively. The exercised animals ran only once a week and the trained animals were run twice a day, four days a week thereafter for eleven weeks. The running schedule allowed a gradual training overload. Equivalent physical work output was assigned between exercised (EAE and EAN), and trained (TAE and TAN) groups during all running sessions. Muscle and liver glycogen concentration was measured as well as muscle fiber glycogen depletion pattern immediately following an acute test (acute situation) and two days following a performance test to exhaustion (chronic situation). Muscle enzymes (phosphorylase and hexokinase) were assayed in the latter condition. In spite of equivalent physical work output, training caused a slightly higher increase in the anaerobically trained animals for hexokinase activity in vastus lateralis white (VW), vastus lateralis red (VR) and soleus (SOL) muscles (57% ($p<.05$), 75% ($p<.05$) and 40% respectively) as compared to aerobically trained animals (20%, 34% and 27% for VW, VR and SOL respectively) over control values. Muscle glycogen levels were

not significantly reduced after the acute test as compared to the age-matched sedentary control. Glycogen depletion patterns in the different muscle fiber types seemed related to work intensity rather than duration. Training led to a 2.4-and 3.2-fold increase in running times for TAE and TAN respectively; the latter group 3.7 times longer than the former one.

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CHAPTER I

INTRODUCTION

The intricate factors related to muscular fatigue have led investigators to elucidate the causes leading to a decrement in work capacity. Considerable evidence tends to support the hypothesis that depletion of carbohydrate stores can play a significant role in the development of muscular fatigue during physical work (Baldwin et al., 1975, Costill et al., 1973, Gollnick et al., 1973).

Depending on work conditions, selective depletion of muscle fiber glycogen stores may occur (Baldwin et al., 1973, 1973a, Gollnick et al., 1973a, 1974, Hermansen et al., 1967, Costill et al., 1971, Gillespie et al., 1974). Therefore, glycogen depletion specific to exercise intensity and duration has been shown to be related to decreased performance times in either interval sprint types of work or endurance running.

It is the purpose of this study to determine whether chronic exercise training of a sprint or endurance nature will differentially affect the total glycogen depletion and pattern in skeletal muscle and liver.

With regard to the metabolic events leading to an activation of glycolysis, it is suggested on one hand that aerobic training might induce a decrease in phosphorylase activity (Baldwin et al., 1973) as opposed to substantial increase (Huston et al., 1975) and on the other hand anaerobic training does not seem to cause any

changes in phosphorylase activity (Staudte et al., 1973) but the findings of Saubert IV et al., 1973, indicated a significant increase in soleus muscle. Such a discrepancy may be explained by the duration of the training period, the muscle analyzed and/or the method used. However, hexokinase activity seems to increase in both types of exercise training with changes most marked in the muscle fibers recruited (Staudte et al., 1973, Baldwin et al., 1973a, Holloszy et al., 1971). Exhaustion, though, is reported to cause a decrease in hexokinase activity (Huston et al., 1975, Barnard and Peter, 1969).

Since adaptations in hexokinase and phosphorylase with different types of training is still unclear, the chronic adaptation in these metabolic markers will be determined to help explain changes in glycogen depletion patterns.

In order to clarify whether alterations in the glycogen depletion pattern is intensity-duration specific and not a function of differences in total work output, the total work output in the two training groups will be kept equivalent.

In view of the limitations imposed by the substrates involved in glucose phosphorylation, the enzymes phosphorylase and hexokinase are believed to offer some insight in this training program analysis. The periodic acid Schiff (PAS) stain was used to evaluate the differential glycogen depletion pattern of muscle fiber types which were classified histochemically (myosin ATPase, NADH diaphorase, α-glycerophosphate dehydrogenase) as fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG) or slow-twitch oxidative (SO) according to the system of Peter et al., 1972. White vastus lateralis

red vastus lateralis and soleus muscles were selected to represent primarily FG, FOG and SO fibers respectively.

Statement of the problem

This study is designed to compare the effect of aerobic and anaerobic training on glycogen depletion in liver and in different types of rat muscle fibers when physical work output is equivalent. Enzyme activities of hexokinase and phosphorylase were used to evaluate the metabolic potential of glucose phosphorylation and glycogenolysis respectively.

Limitations

Extrapolation of this study to human beings must be made with reserve since the species differences include factors such as fiber type distribution, developmental and metabolic patterns of the muscles studied.

Subjective evaluation of PAS staining using an arbitrary scale as well as fiber typing (intensity of staining for ATPase, α-GPDH and NADH-diaphorase activities).

Definition of terms

Acute situation: condition in which the animals were sacrificed immediately following cessation of the acute test.

Aerobic training: continuous running at 40 m/min and 15%grade

Anaerobic training: running at 80 m/min and 15% grade with work periods (15 sec) interspersed with rest periods (30 sec).

Chronic situation: condition in which the animals were sacrificed at least two days following the last training session.

Control 1 (CON1): sedentary animals (N = 7) sacrificed one week after arrival at approximately 5-6 weeks of age.

Control 2 (CON2): sedentary animals (N = 7) sacrificed at the end of the experiment and handled every day.

Equivalent total work output: total work periods corresponding for the anaerobic group twice as fast but twice as short as compared to the aerobic group e.g. 10 repetitions of 15 sec work bouts alternated with 30 sec rest periods (total work time: 2:30 min) at 80 m/min is equivalent to 5 min (continuous work) at 40 m/min.

Exercised aerobic (EAE): animals (N = 12) submitted to progressive training in preparation for aerobic work (40 m/min, 15% grade) and trained once a week thereafter for eleven weeks. This group was subdivided in two groups: EAEC (n = 6) chronic situation and EAEA (N = 6) acute situation.

Exercised anaerobic (EAN): animals (N = 12) submitted to progressive training in preparation for anaerobic work (80 m/min, 15% grade) and trained once a week thereafter for eleven weeks. This group was subdivided in two groups EANC (N = 6) chronic situation and EANA (N = 6) acute situation. Each one of these animals were yoked with an animal in group EAE and performed an equivalent total work during exercise sessions.

Fast-twitch glycolytic (FG): muscle fibers with high glycolytic

capacity, low oxidative capacity, high ATPase activity and fast-twitch contractile properties (high α-GPDH, low NADH-D and high ATPase). This nomenclature is based on the classification of Peter et al., 1972.

Fast-twitch oxidative glycolytic (FOG): muscle fibers with high glycolytic capacity, high oxidative capacity, high ATPase activity and fast-twitch contractile properties (high α-GPDH, high NADH-D and high ATPase).

Slow-twitch oxidative (SO): muscle fibers with low glycolytic capacity, high oxidative capacity, low ATPase activity and slow-twitch contractile properties (low αGPDH, high NADH-D and low ATPase).

Trained aerobic (TAE): animals (N = 12) submitted to progressive training in preparation for aerobic work (40 m/min, 15% grade) and trained twice daily four days a week thereafter for eleven weeks. This group was subdivided in two groups TAEC (N = 6) chronic situation and TAEA (N = 6) acute situation.

Trained anaerobic (TAN): animals (N = 6) submitted to progressive training in preparation for anaerobic work (80 m/min, 15% grade) and trained twice daily four days a week thereafter for eleven weeks. This group was subdivided in two groups TANC (N = 6) chronic situation and TANA (N = 6) acute situation. Each one of the animals were yoked with an animal in group TAE and performed an equivalent total work output during training sessions.

Abbreviations

α-GPDH: α-glycerophosphate dehydrogenase (EC 1.1.99.5)

ATP: adenosine tri-phosphate

ATPase: adenosine tri-phosphatase (EC 3.6.1.3)

°C: degree Celsius

cm: centimeter

CON: control

CPM: counts per minute

DPM: disintegration per minute

EAEA: Exercised AErobic Acute

EAEC: Exercised AErobic Chronic

EANA: Exercised ANaerobic Acute

EANC: Exercised ANaerobic Chronic

FFA: free fatty acid

FG: fast-glycolytic

FOG: fast-oxidative glycolytic

FT: fast-twitch

g: gram

HK: hexokinase (EC 2.7.1.1)

m: meter

mg: milligram

μ: micron

μmole: micromole

min: minute

mmole: millimole

NADH-D: reduced nicotinamide adenine dinucleotide diaphorase

PAS: periodic acid Schiff

Pase: Phosphorylase (EC 2.4.1.1)

sec: second

SEM: standard error of the means

SOL: soleus muscle

ST: slow-twitch

TAEA: Trained AErobic Acute

TAEC: Trained AErobic Chronic

TANA: Trained ANaerobic Acute

TANC: Trained ANaerobic Chronic

U: (international) Unit or μmole/min

$\dot{V}O_2$ max: maximal oxygen consumption per minute

VR: vastus lateralis red muscle

VW: vastus lateralis white muscle

CHAPTER II

REVIEW OF LITERATURE

1. Aerobic work: glycogen metabolism and muscle glycolytic enzymes

1.1 Glycogen utilization

The muscle contraction process is known to be energy dependent, with the direct energy source, ATP, being largely supplied by glycolysis and mitochondrial oxidative phosphorylation during a moderate intensity exercise. In skeletal muscle, glycogen reserves are acting as the main carbon substrate for energy production especially at the onset of exercise.

Based on these facts, Gollnick et al., 1974, were interested in looking at the glycogen depletion pattern in human skeletal muscle fibers following exercise at varying work intensities. Their results demonstrated that for work loads requiring less than 100% of maximum oxygen consumption ($\dot{V}O_2$ max), slow-twitch (SO) fibers were the first to lose glycogen. Even during light exercise (30% $\dot{V}O_2$ max) the rate of oxidation of substances other than glycogen like blood glucose, plasma free fatty acids (FFA) and intramuscular lipids was insufficient to satisfy exercise energy needs and glycogen loss in ST fibers was observed at a rather early stage after the onset of exercise. As exercise proceeded, subsequent ST fibers became glycogen depleted suggesting additional recruitment of motor units. Even though ST

fibers were the first to be depleted of their glycogen stores at high workloads (83% VO_2 max), fast-twitch (FT) fibers played an important role in handling higher tension developed by muscle as well as higher energy demands. This is in total agreement with a previous study of Gollnick et al., 1973.

A similar finding was noticed in a study done by Costill et al., 1973, where prolonged running led to selective and marked glycogen depletion of ST fibers with only a minor reduction in FT fibers. The average oxygen uptake was 83% of VO_2 max including down- and uphill conditions. However, of some interest was the observation that the situation is more complex than simply a direct relationship between total muscle glycogen and ability to perform long distance running. Even with some glycogen left in their muscle fibers, the runners could not keep up with the pace near the end of the 30 kilometer race. One must be careful in its generalization from biopsy samples that even a selective glycogen depletion in some fibers has occurred, it is not necessarily a reflection of the total glycogen consumption or the metabolic reserves of the working fibers. Their data indicate that a very large portion of man's muscles mass is engaged in running but a detailed information on the relative rate of glycogen depletion in different muscle groups is lacking.

In a study of glycogen metabolism during exercise in men, Bergstrom and Hultman (1967) observed that the working capacity of skeletal muscle (vastus lateralis) was dependent in its glycogen stores which fell steadily during exercise. In sampling muscle tissue with the biopsy technique at different time intervals, they noticed a semi-logarithmic depletion pattern with a rapid glycogen breakdown

during the first minutes of exercise leading to a rapid increase in lactate production. However, a slower breakdown of glycogen occurred only when its muscle content was very low and as a consequence an increased glucose output from the liver was recorded. Also, during glucose infusion, glycogen consumption was significantly lower. However, muscle glycogen was responsible for the greater part of energy production, even when blood sugar level was high. They also found a high correlation ($r = 0.91$) between the fall in muscle glycogen and pulse rate suggesting that the rate of glycogen utilization is related to the relative workload rather than the absolute one.

As suggested by Baldwin et al., 1975, the level of physical training might be of significance importance. They analyzed carbohydrate depletion in liver and in the three different types of skeletal muscle of exercise-trained and untrained rats. They found that the rate of liver glycogen depletion was lower and that the remaining glycogen stores in all three muscle fiber types were significantly higher in the trained than the untrained animals. It appears reasonable that because of an increased oxidative capacity, the muscle fibers can derive more energy from fat oxidation leading to a protective effect of exercise-training on muscle glycogen stores.

Similarly, Fitts et al., 1975, were interested in the relationship between the respiratory capacity of muscles and the depletion of carbohydrate stores during submaximal exercise. They observed that carbohydrate utilization during an aerobic-type exercise was inversely correlated with the concentration of mitochondria in the animal's leg muscles. It seems likely that the animals with a higher concentration of mitochondria utilized proportionally more fat and therefore leading

to a sparing-effect of the carbohydrate stores both in liver and in exercising muscles during submaximal work.

It is believed that the constituents of the diet play a significant role in muscle metabolism. In an attempt to investigate glycogen storage in the different fiber types of human skeletal muscle and its depletion pattern during exercise under different diets, Gollnick et al., 1972, looked at muscle biopsies performed on four subjects working at an average intensity of 74% of their aerobic power. With a mixed and carbohydrate diets, they observed a higher glycogen content in the FT fibers than in the ST fibers before exercise and a greater reduction in muscle glycogen occurred in the ST fibers after exercise. However, of some interest was the finding that when muscle glycogen was lowered due to a high fat-protein diet, this glycogen reduction was greater in the FT fibers. In the latter condition, when the glycogen stores of the ST fibers were reduced to a point where they could not meet the metabolic requirements of these fibers, the recruitment pattern was shifted toward the FT fibers in order to produce the desired contractile force and keep up with the working intensity. It is rather important to provide the proper diet in order to meet adequately the demanding metabolic rate during prolonged exercise.

Analogously, Costill et al., 1971, were interested in muscle glycogen utilization during prolonged exercise on successive days. They noticed that in muscle biopsies of the vastus lateralis, the greatest glycogen utilization occurred in the first ten mile run as compared to the second and third one. One explanation to that observation could be the insufficient rest period (24 hours) between each

run which did not allow for a full replenishment of the initial glycogen stores. To compensate for the diminution in glycogen utilization during the second and third run, an increase in lipid catabolism would enable the subjects to meet energy requirements of prolonged running. The dietary carbohydrate sources as reported by Gollnick et al., 1972, played a significant role in muscle glycogen repletion; the moderate amount (40-50%) of carbohydrate sources may explain the reason on incomplete muscle glycogen replenishment in three subjects even after five days of rest.

The importance of the initial glycogen content in different muscle fibers has led Gillespie et al., 1970, to question the general consensus that glycogen concentration is higher in white than red muscle. It was generally observed that red fibers, with a minimum number of intermediate fibers, stained more intensely than white fibers using PAS stain suggesting that the intermediate fibers may be responsible for the frequently demonstrated higher glycogen content in white muscle.

1.2 Muscle glycolytic enzymes

In order to investigate the mechanisms involved during adaptation to endurance training, Baldwin et al., 1973a, compared the levels of glycolytic enzymes in different types of skeletal muscle in exercise-trained and sedentary rats. Among several enzymes analyzed, phosphorylase level showed a small but consistent 20% decrease (105 and 81 μmoles/min/g for sedentary and exercised animals respectively). On the other hand hexokinase provided the most significant adaptation in oxidative muscle fibers reflecting a preferential utilization of these fibers during prolonged relatively slow running. The red quadriceps

showed a 173% increase for the exercising animals as compared to the sedentary one (4.1 and 1.5 μmoles/min/g muscle respectively), soleus muscle indicated a 50% increase (2.39 and 1.37 μmoles/ min/g respectively) and the white quadriceps showed only a 30% increase (0.75 and 0.58 μmole/min/g muscle in the exercising and sedentary groups respectively).

The same trend was found in the Holloszy et al., 1971, study where phosphorylase activity was not significantly altered in gastrocnemius muscle after a low intensity training period, whereas hexokinase activity was reported twice as high in the leg muscles of the treadmill runners as in those of the sedentary controls.

Similarly, Huston et al., 1975, investigated the influence of diet, training, rested and exhausted states on the in vitro activity of muscle glycolytic enzymes. They found a significant 20% increase in phosphorylase activity in gastrocnemius muscle of the trained rats (30.5 and 36.6 μmoles/min/g of tissue for untrained-rested and trained-rested rats respectively). Of some interest was the observation that exhaustion increased phosphorylase activity in untrained rats to a value equivalent to the trained one at rest (36.7 μmoles/min/g). On the other hand, hexokinase showed a maximal enzyme activity 73% greater in the trained-rested as compared to the untrained rats (1.96 and 1.13 μmoles/min/g respectively). However, exhaustion lowered enzyme activity about 13%. Unfortunately, the findings of this study could not be attributable to a particular fiber type composition since they used the whole gastrocnemius muscle.

Kowalski et al., 1969, looked at phosphorylase activity in rat quadriceps muscles after a training period of running and weight lifting.

The former exercise condition induced a greater increase in phosphorylase activity than the latter one, but the tinctorial determination leading to a qualitative estimation of the phosphorylase activity made it difficult to compare with other studies.

Similarly, Hickson et al., 1976, evaluated qualitatively phosphorylase activity using a photometer which measured the percentage of light passing through the central portion of a single muscle fiber previously stained for that enzyme. They found significant decrease in phosphorylase staining intensities in the soleus, gastrocnemius and plantaris muscles with sprint training of eight weeks. After the thirty-seventh day of training, the animals were completing eight bouts of exercise consisting of six repetitions of 10 sec of running at 99 m/min alternated with 40 sec of rest. They noticed also glycogen supercompensation in the white muscle regions which consisted mainly of fast-twitch fibers, those recruited mainly in sprinting regimen.

Hexokinase has been the key enzyme analyzed by Barnard and Peter, 1969, in male and female guinea pigs after an aerobic training. The fact that skeletal muscle hexokinase increased with training as well as with a single exhaustive bout of exercise led the investigators to question its correlative significance with running endurance. They found that muscle hexokinase activity is dependent upon the muscle type, the intensity and length of training without being directly related to the training level. Harri et al., 1975, reported a 16% increase (1.09 and 1.26 µmoles/min/g of muscle tissue in control and trained rats respectively) in hexokinase activity in response to training (swimming) in gastrocnemius muscle.

2. Anaerobic work: glycogen metabolism and muscle glycolytic enzymes

2.1 Glycogen utilization

Gollnick et al., 1973, studied the glycogen depletion pattern in human vastus lateralis after short high-intensity work (150% of subjects' aerobic power). There was a linear decline in muscle glycogen as a function of the number of sprint bouts. Due to the nature of exercise, the fast-twitch (FT) fibers were the first to become depleted of their glycogen stores whereas the lower glycolytic potential of the ST fibers may have prevented a rapid rate of glycogen utilization. As a consequence, the subjects reached exhaustion after six exercise bouts even with a 10 min rest period between each bout. A reduction in the number of contracting fibers, due to glycogen loss, was suspected to have diminished the contractile force necessary to sustain the heavy exercise condition.

In a prolonged severe exercise to exhaustion, Hermansen et al., 1967, showed the importance of muscle glycogen as measured by the muscle biopsy technique. The steeper fall in glycogen level was reported to happen during the first exercise period lasting 20 minutes. They came to the conclusion that the inability to perform exercise was due to a drastic decrement in muscle glycogen level even though the combusted carbohydrate, based on respiratory quotient (RQ) and oxygen uptake, remained fairly constant. A point to keep in mind is that glycogen was determined only in the lateral portion of the quadriceps muscle whereas RQ measurement was determined for the whole body. Nevertheless, the initial muscle glycogen content seemed to be

a determinant factor in prolonged exhaustive work. Similarly, Hultman, 1967, stressed the point that if one is to perform heavy work, glycogen must be there before he starts. One of the best ways to augment glycogen stores is to replenish them with a high carbohydrate diet following heavy work.

Different tensions applied during isometric contraction may deplete glycogen stores to varying degrees. Gollnick et al., 1974, found that a selective glycogen depletion pattern indicative of a differential recruitment of muscle fibers occurred in isometric exercise of varying intensity (% of maximum voluntary contraction, MVC). At low isometric tensions (less than 20% MVC), only ST fibers were depleted of their glycogen whereas FT fibers retained their PAS staining even after nearly 30 minutes of sustained exercise (isor ic tension). However, at intensity higher than 20% MVC, it was interesting to note that FT fibers were the only fibers to become PAS negative and ST fibers retained PAS staining similar to the values found at rest suggesting that fiber recruitment is dependent on the tension developed by contracting muscle. Thus, during early stage of multiple contractions at relative high tensions a large number of fast motor units are recruited.

A 32% higher glycogen content of the trained leg over the untrained one at rest was reported by Piehl et al., 1974. This rather important increase is attributable to a concomitant 18% increase in hexokinase activity and a 36% increase in glycogen synthet ctivity in the exercising leg as compared to the other one. They explained that hexokinase would enhance glycogen synthesis by augmenting the glucose-6-phosphate pool in muscle.

2.2 Muscle glycolytic enzymes

The importance of glucose entry into the glycolytic pathway interested Saubert IV et al., 1973, who studied the activity of the rate-limiting enzymes in the regulation of the glycolytic flux during anaerobic work. The rats were able to run eighteen 30 sec sprints (80.5 m/min) interposed with 30 sec rest periods at the end of a 11 week training program. Phosphorylase activity was analyzed in gastrocnemius (red and white portion), red vastus lateralis and soleus muscles. A significant increase with training appeared only in soleus muscle in spite of its lowest phosphorylase activity in the muscles studied.

In a training program which stressed the anaerobic capacity of laboratory rats, Staudte et al., 1973, studied the main enzymes involved in the energy-supplying metabolism both in slow and fast muscles. Phosphorylase level did not change significantly in rectus femoris after a 21 day training period whereas increases were induced in hexokinase activity (1.0 and 1.47 U/g or µmoles/min/g for the control and trained animals respectively). The same trend was reported in soleus muscle.

Jobin (1976) found also a significant 94% increase in hexokinase activity (0.42 and 0.79 µmoles/min/g for control and trained animals respectively) after a high intensity (80m/min) training program in gastrocnemius muscle of rats.

CHAPTER III

METHODOLOGY

1. Animal care

Sixty-two male Wistar rats (Woodlyn Farms, Guelph, Ontario) were randomly assigned to five groups: I sedentary control (N = 14), II exercised aerobic (N = 12), III exercised anaerobic (N = 12), IV trained aerobic (N = 12), and V trained anaerobic (N = 12). Each animal was housed in a separate cage and fed ad libitum a conventional diet of laboratory rat chow (see Appendix F for content). Water was changed regularly, cages and water bottles were washed every week.

All animals were given one week of familiarization with their new environment: food, experimenters, 12 hour day-night cycle (dark: 7:00 AM to 7:00 PM), temperature and cages which were rotated in order to expose each animal to different cage positions. The experimenters progressively tamed the rats by handling them every day. Each animal was identified by a code system using notches on ears; each notch on the upper left ear corresponded to thirty units, lower left to three units, upper right to ten units and lower right to one unit.

A sick animal was isolated and treated properly, when it was too serious the animal was anesthetized with ether and sacrificed. Individual care was given especially after each training session, each rat being thoroughly checked for injuries, properly treated with disinfectant and well dried with a towel before returning to its cage.

2. Training program

At the end of the familiarization week, the animals in group II and IV were exercised aerobically on a motor-driven treadmill (Quinton rodent treadmill) consisting of a wide endless belt on rollers whereas those in groups III and V were exercised anaerobically. A plexiglass frame was placed over the rolling belt and consisted of ten compartments (75 × 10 × 10 cm) with a shock grid embedded at the rear of each compartment. Electrical stimulation (50 volts) was chosen as motivational approach during training.

After eleven days of running twice a day during the last eight days with week-ends and Wednesdays off, the aerobic groups (II and IV) reached the 40 m/min-15% grade criterion and the anaerobic groups (III and V) reached the 80 m/min-15% grade criterion. The animals were brought progressively to their own criterion as can be seen in Appendix E.

Once the pre-training session was completed, groups II and IV were able to run consecutively five minutes at 40 m/min and 15% grade while groups III and V were able to perform an equivalent total work output i.e. ten intervals (15 sec on and 30 sec off) at 80 m/min and 15% grade. Thereafter the exercised groups (II and III were run only on Wednesdays to maintain their running ability without any significant training effect.

The animals in groups IV and V were continually trained as to their respective training regimen for nine more weeks totalizing three months of physical training with an equivalent work output performed in each group. Throughout the training period, the running schedule was adapted in order to overload their aerobic and anaerobic systems

respectively. In order to assign the equivalent total work output, all the rats in group IV were yoked with those in group V on a random basis; the same procedure was applied between the exercised groups (II and III).

The two daily training sessions were seven to eight hours apart, the morning one being held between seven and nine o'clock and the afternoon one between three and five o'clock. They were trained four days a week (Monday, Tuesday, Thursday and Friday). However, the injured runners were properly treated and left in their cage for the following training session. A recovery period lasting more than two days led to the exclusion of the animal from the study.

3. Experimental design

On the day of arrival, the animals were assigned to a cage as to their order of presentation in the delivery box. The next day, the groups were formed at random from the total number of animals (the figures appearing in table I correspond to the number of animals at the end of the study) using a computer random number list which determined the assignment of an animal to a particular group. One week later each rat was identified with a code using notches on ear as previously described.

As shown in the experimental design, group CON was divided at random into two subgroups CON1 and CON2, the former (7 animals) being sacrificed at the end of the familiarization week and the latter (7 animals) 13 weeks later. Each of the groups II (EAE: exercised aerobic), III (EAN: exercised anaerobic), IV (TAE: trained aerobic), V (TAN:

Table I Experimental design

GROUPS		SUB-GROUPS	number of animals sacrificed at different times after arrival		TOTAL
			10 days	13 weeks + 2 days	
I	CON	CON1	7	–	7
		CON2	–	7	7
II	EAE	EAEA	–	6	6
		EAEC	–	6	6
III	EAN	EANA	–	6	6
		EANC	–	6	6
IV	TAE	TAEA	–	6	6
		TAEC	–	6	6
V	TAN	TANA	–	6	6
		TANC	–	6	6
	TOTAL		7	31	62

trained anaerobic) were divided at the end of the training period on a random basis, into two subgroups consisting firstly of an acute (A) situation which looked at glycogen depletion in liver and different types of muscle fibers after a performance test, and secondly of a chronic (C) situation which included the analysis of enzymatic adaptation at least two days after the last training session which consisted of a performance test to exhaustion.

4. Performance tests

In order to evaluate their running performance, a test to exhaustion was administered to the chronic groups (EAEC, EANC, TAEC and TANC) during their last training session (two days before sacrifice). The aerobic groups (EAEC and TAEC) ran continuously at 40 m/min on the treadmill at 15% incline until exhaustion which was determined when the rat, placed on his back, could not roll over. The anaerobic groups (EANC and TANC) ran at 80 m/min and 15% incline, for intervals of 15 sec alternated with 30 sec of rest, until exhaustion (same criterion as the aerobic animals). The total running time and the sum of work periods determined the performance level of aerobic and anaerobic groups respectively.

The performance test for the acute groups (EAEA, EANA, TAEA, TANA) consisted of a five minute continuous run at 40 m/min and 15% incline for the aerobic groups (EAEA and TAEA) and of ten repetitions of 15 sec of running at 80 m/min and 15% grade alternated with 30 sec of rest for the anaerobic groups (EANA and TANA). The purpose of that

test was to bring about a significant glycogen depletion in muscle fibers without including an exhaustive type of performance in either groups. The rats were sacrificed by decapitation immediately after their run.

5. Environmental conditions

The laboratory room was part of a centrally heated and air conditioned building. The room was also well aerated and temperature kept constant at 21 °C (± 1°). The rats were trained in dark with a dim light for the experimenter and the training ti: :e kept constant throughout the experiment.

6. Sacrifice procedures and tissue sampling

The animals were sacrificed by decapitation (Guillotine), rapidly exsanguinated, liver quickly removed as was the skin of the right hind leg exposing the quadriceps muscles. The vastus lateralis was then excised, freed from connective and adipose tissue and separated in its white and red portion. Subsequently, the soleus muscle was excised and freed from connective tissue. The liver and muscle samples were blotted, weighed and immediately frozen in isopentane, cooled in liquid nitrogen and stored in a deep freezer (-60°C) until subsequent enzyme analysis. The muscles of the left leg were subsequently excised frozen and stored as above and used for biochemical and histochemical purposes.

7. Procedures for enzyme assays

7.1 Homogenization procedures

The muscle tissue was ground into a fine powder in a mortar containing liquid nitrogen. Grinding procedures were done in a cold room (5°C). Once the tissue was ground, more liquid nitrogen was added and a measured volume of phosphate buffer was poured on top of the boiling liquid nitrogen resulting in the formation of ice crystals which were ground to a very fine powder together with the tissue powder. The whole content was transferred to a conically shaped centrifuge tube which was previously sitting on dry ice.

The ground muscle mixed with the phosphate buffer was allowed to thaw in an ice bath and then was centrifuged at 1500×g for five min (0°C). The supernatant was poured into a test tube and enzyme analysis was performed immediately. The muscle of the left leg were analyzed for phosphorylase activity and the muscles of the right leg for hexokinase activity; the reason being that a slightly different buffer solution was utilized during the grinding process as can be seen in Appendix B.

7.2 Phosphorylase enzyme assay

Muscle phosphorylase enzyme was assayed following the Russell et al., 1970, technique. Tubes containing 0.1 ml of substrate solution as well as those containing muscle extract were incubated and shaken for 15 min at 30°C. 0.1 ml of the muscle extract sample was added to the substrate tubes which were shaken during the precisely timed

enzyme reaction (5 min). At the end of the five minute enzyme reaction, 1.0 ml of ice-cold stop solution of trichloroacetic acid was added directly to the liquid surface and mixed thoroughly. The assays were performed in triplicate.

Cold 95% ethanol, 2 ml, was added and the samples were allowed to set for 20 min in order to precipitate glycogen; then centrifuged at 2000×g for 10 min. The supernatant fluids were decanted and discarded in a radioactive waste bottle. The precipitates were washed with cold 66% ethanol, 2 ml, and centrifuged at 2000×g for 10 min. The same procedure was repeated three times. The glycogen pellets were finally resuspended in 0.5 ml cold 66% ethanol and washed into counting vials with 1 ml of distilled water. The vials were taken to dryness in a vacuum oven at approximately 40°C for at least 20 hours. One ml of NCS (basic solubilizing agent: Amersham/Searle) was added and the vial well shaken before adding 15 ml of toluene containing 4 g PPO and 50 mg of POPOP per liter. The samples were counted in a liquid scintillation counter (Nuclear Chicago Mark III) equipped with a program designed to give maximum counting efficiency and lowest background. Blank samples were run, the muscle extract being added after the stop solution of trichloroacetic acid.

The substrate solution was composed of 1 g of glycogen and 32 mmoles/of G-1-P (dipotassium salt) including the uniformly labeled G-1-P^{14}C (7.49×10^7 DPM/mmole) in 50 ml of the buffer solution used for homogenization, pH 6.1 (sufficient for approximately 500 assays). 0.1 ml of the substrate solution was pipetted in small test tubes and kept frozen until enzyme assays.

7.3 Hexokinase enzyme assay

A series of 20 muscle samples selected at random were homogenized, according to the technique of Joshi et al., 1966, and analysed immediately following centrifugation (see Appendix B). ATP and NADH were always freshly prepared. To eliminate background activity, the content of the reference cell was the same as the sample cell except for the specific substrate, then eliminating the non-specific reactions from the total enzymatic activity of the muscle extract or homogenate.

The enzyme assays began with a 15 min incubation period at 37°C before the reaction reached an equilibrium in the spectrophotometer (UNICAM SP 800) cell holder which was kept at 37°C by means of a circulating bath. Then the hexokinase reaction was started by the addition of the specific substrate ATP and the cuvette was mixed by inversion. Direct recording was done over a fifteen minute period allowing for a constant rate of NADPH formation. Details of the reaction can be seen in Appendix B.

7.4 Glycogen assays

Muscle and liver glycogen was measured using Lo et al., 1970, technique for small tissue samples. The absorbance was read at 630 nanometers using a Gilford (300-N) micro-sample spectrophotometer.

8. Histochemical technique

Comparative glycogen depletion in different fiber types was evaluated in the vastus lateralis white, red and the soleus muscles using on one hand serial 16 μ sections for periodic acid Schiff (PAS) stain (Karlsson et al., 1970) and on the other hand serial 10 μ sections for myosin ATPase (Guth and Samaha, 1969), NADH diaphorase (Dubowitz and Brooke, 1973) and α-GPDH (Wattenberg and Leong, 1960). Frozen serial cross sections were cut at -22°C using a microtome in a cryostat. The sections were mounted on cover glasses and air dried for at least 10 hours.

9. Data analysis

9.1 Calculation of phosphorylase activity

Its activity is reported in micromoles of labeled product formed per minute per gram of tissue (wet weight): μmoles/min/g. The following formula was used to calculate Pase activity:

$$\text{RATE} = \frac{c/t}{C \times (W \times v/V)} \quad \text{in } \mu\text{moles/min/g}$$

where: c = counts per min (CPM) in scintillation vial

t = incubation time (min) during enzyme assay

C = counts per minute (CPM)/μmoles

W = muscle weight in gram

v = volume (ml) of muscle extract added during enzyme assay

V = volume (ml) of buffer added during homogenization

9.2 Calculation of hexokinase activity

Its activity is reported in micromoles of NADPH formed per min per gram of tissue (wet weight): µmoles/min/g. The following formula was used to calculate hexokinase activity:

$$\text{RATE} = \frac{\Delta\text{O.D.} \times V \times 10^3}{6220 \times (W \times v_2/v_1)} \quad \text{in µmoles/min/g}$$

where:

ΔO.D. = change in optical density at 340 nm

V = total volume (ml) in cuvette

10^3 = transformation factor

6220 = extinction coefficient of NADPH at 340 nm

W = muscle weight in grams

v_2 = volume (ml) of muscle extract added during enzyme assay

v_1 = volume (ml) of buffer added during homogenization

9.3 Statistical analysis

The data of enzyme activity, body weight, glycogen values were analyzed using a one-way analysis of variance (ANOVA) (Winer, 1971). Scheffé multiple comparison of means (Winer, 1971) post hoc procedures were used to evaluate the significant differences between groups (DERS program documentation: ANOV15). In all statistical analyses, an $\alpha = 0.05$ or smaller was a requirement for significance.

Since each cell in the analysis of variance (ANOVA) has an equal number of animals, Scheffé's multiple comparison of means is a legitimate statistical procedure even though homogeneity of variance is not reached (Winer, 1971).

CHAPTER IV

RESULTS

The results are summarized in the form of tables and figures which represent mean values and standard errors of the means (SEM) for each group. Raw data can be found in Appendix C and analyses of variance with Scheffé multiple comparison of means in Appendix D.

1. Body weight

Body weight followed a regular pattern in all groups throughout the experiment with no difference between groups at the beginning of the training program (Figure 1). The trained groups (TAE and TAN) showed a significant lower body weight at the end of the experiment (Appendix D-1) as compared to the sedentary group (CON).

2. Performance test

The trained groups, TAN and TAE, performed sifnificantly longer than the exercised groups, EAN and EAE, respectively. Their work times were 67:30 (± 9:49 min), 21:00 (± 2:52 min), 18:17 (± 5:27 min) and 7:47 (± 0:47 sec) for groups TAN, TAE, EAN and EAE respectively (Figure 2).

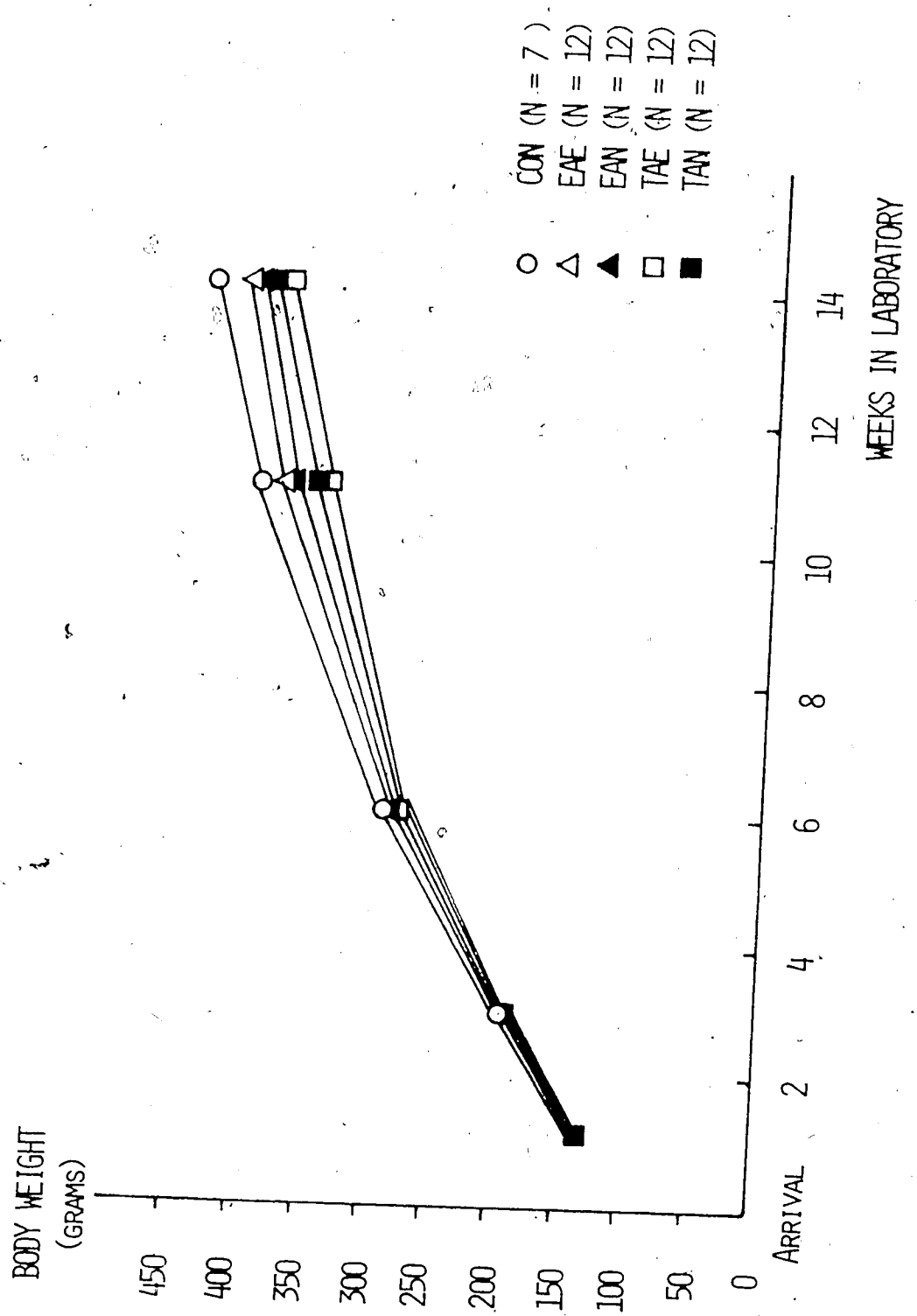


FIGURE 1 PROGRESSION OF RAT BODY WEIGHTS FOR THE DIFFERENT EXPERIMENTAL GROUPS

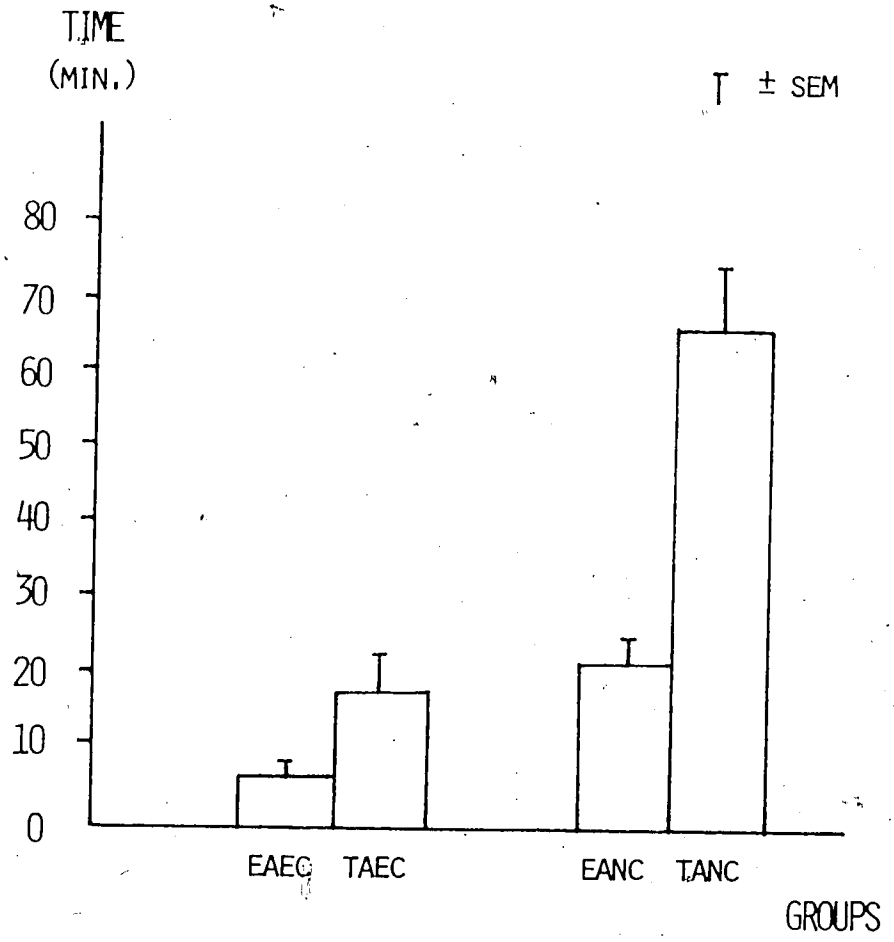


FIGURE 2 PERFORMANCE TEST TIMES ON A CONTINUOUS TASK (40 M/MIN, 15% GRADE) FOR THE AEROBIC GROUPS (EAEC, TAEC) AND AN INTERVAL TASK (80 M/MIN, 15% GRADE, 15/30 SEC WORK/REST RATIO) FOR THE ANAEROBIC GROUPS (EANC, TANC).

3. Glycogen values

3.1 Muscle glycogen

In the chronic situation, where the rats were sacrificed two days after their last training session, there was no significant difference between the exercised and trained groups for glycogen content (range of means 9.0 to 14.3 mg of glycogen/g of tissue) in vastus lateralis white (VW) muscle (Figure 3), in vastus lateralis red (VR) (range of means 5.8 to 12.6 mg/g) and in soleus (SOL) (range of means 6.2 to 11.4 mg/g).

In the acute situation, where the animals were decapitated immediately after exercise, no significant difference was demonstrated between group means (Appendix D-2, D-3 and D-4).

3.2 Liver glycogen

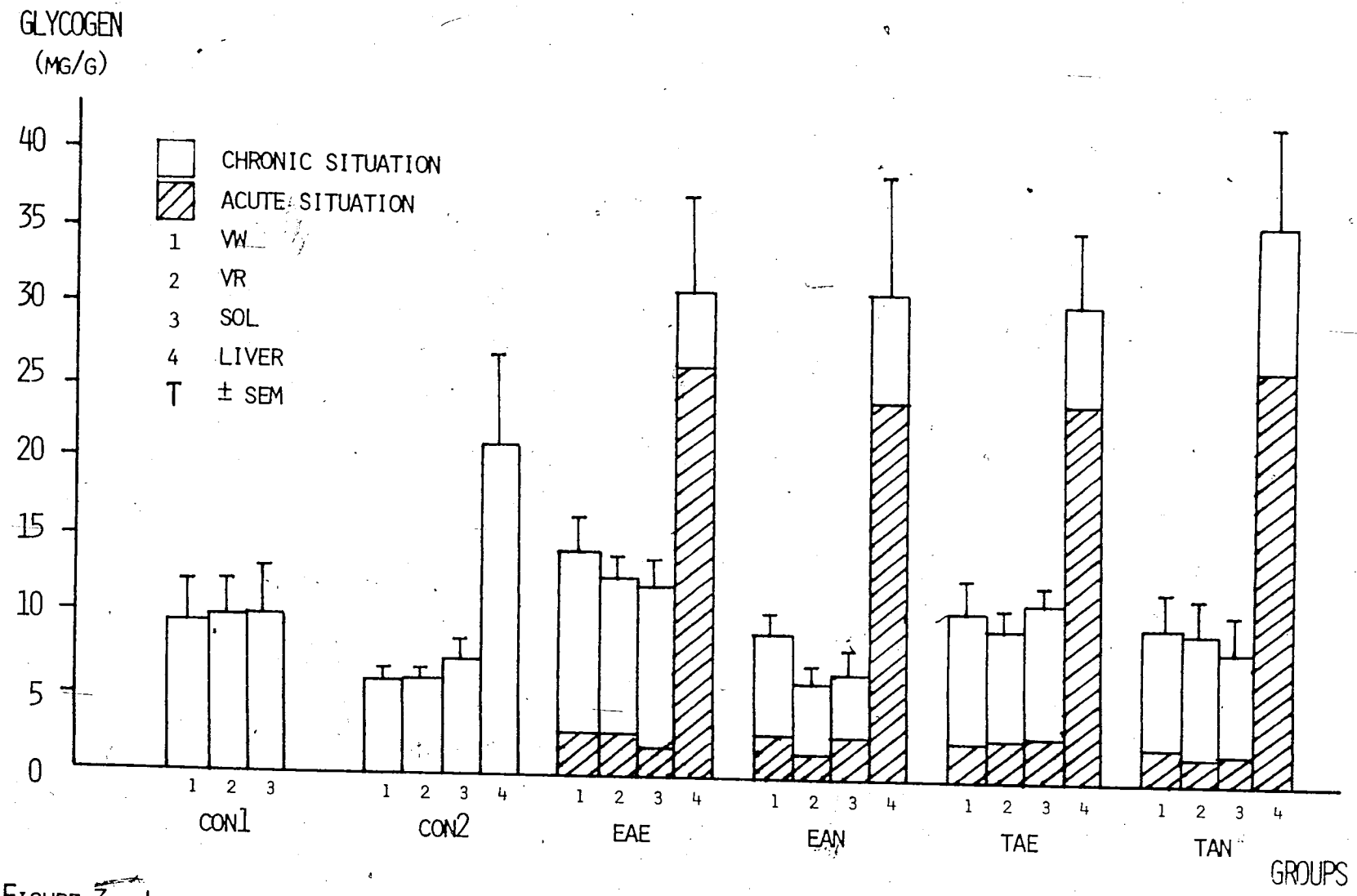


FIGURE 3 LIVER AND MUSCLE GLYCOGEN IMMEDIATELY FOLLOWING A SUBMAXIMAL (ACUTE) EXERCISE AND APPROXIMATELY 2 DAYS FOLLOWING THE LAST TRAINING SESSION (CHRONIC).

Liver glycogen followed a uniform pattern between groups and approached mean control value (20.9 mg/g) after the acute test (25.9, 23.8, 23.8 and 26.8 mg/g for EAEA, EANA, TAEA and TANA respectively) (Figure 3). The analysis of variance (Appendix D-5) failed to show a significant difference between groups in both chronic and acute situations.

3.3 Muscle fiber and glycogen depletion

The main purpose of fiber typing was to verify fiber type composition in each muscle. As represented in Figure 4, vastus lateralis white muscle was predominantly composed of fast glycolytic (FG) fibers (86%, 97%, 87%, 79%, 73% and 71% of total fibers for groups CON1, CON2, EAE, EAN, TAE and TAN respectively) with few fast oxidative glycolytic (FOG) fibers (the remaining percentage). The staining procedures, detailed previously, failed to show slow oxidative (SO) fibers in that muscle. The PAS stain revealed a low percentage of FG fibers depleted of their glycogen stores after the acute test (Table II) (10%, 4%, 6% and 2% of FG fibers depleted of their glycogen stores for groups EAEA, EANA, TAEA and TANA respectively). The FOG fibers were more extensively utilized as judged by a 47%, 65%, 45% and 36% depletion of glycogen stores in groups EAEA, EANA, TAEA and TANA respectively (Table II).

In vastus lateralis red muscle, a somewhat more heterogeneous fiber type distribution was observed, nevertheless most of them were of a fast-twitch nature (Figure 4) (13%, 31%, 41%, 31%, 26% and 47% of FG fibers and 73%, 61%, 55%, 66%, 66% and 49% of FOG fibers in

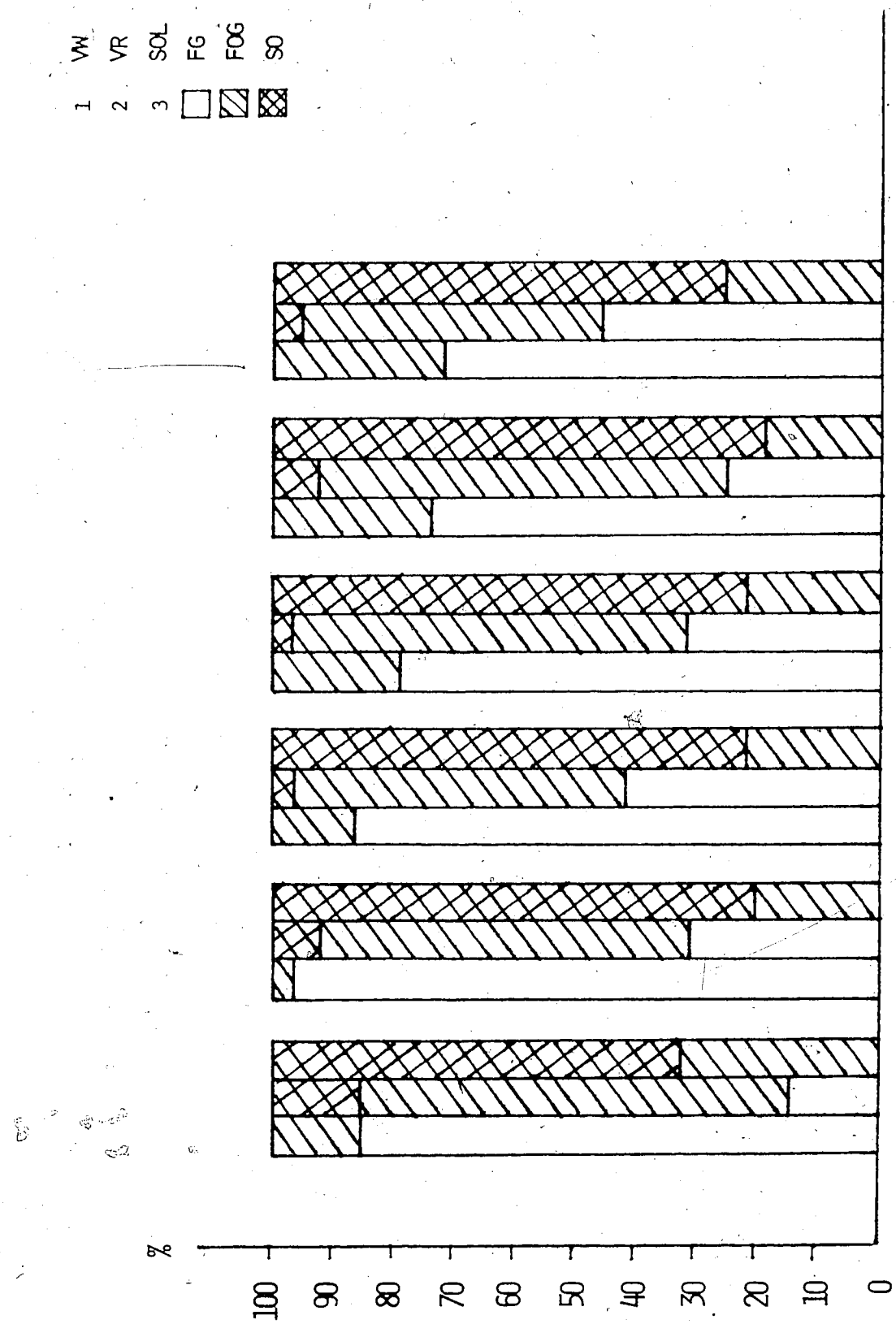


FIGURE 4 PERCENTAGES OF MUSCLE FIBER TYPES

Table II Percentages of muscle fiber types and percentages of fibers completely depleted of glycogen (using PAS staining) (in parentheses) following a submaximal acute exercise.

GROUPS	SKELETAL MUSCLES	FIBER TYPES			FIBERS COUNTED
		FG	FOG	SO	
	VW	86	14	--	1745
CON1	VR	13	73	14	1021
	SOL	--	32	68	1193
	VW	97	3	--	1112
CON2	VR	31	61	8	1206
	SOL	--	20	80	1065
	VW	87 (10)	13 (47)	--	1253
EAE	VR	41 (100)	55 (100)	4 (100)	1514
	SOL	--	21 (97)	79 (100)	1715
	VW	79 (4)	21 (65)	--	1318
EAN	VR	31 (98)	66 (100)	3 (100)	1622
	SOL	--	21 (95)	79 (87)	
	VW	73 (6)	27 (45)	--	
TAE	VR	26 (99)	66 (100)	8 (100)	1796
	SOL	--	18 (97)	82 (100)	2035
	VW	71 (2)	29 (36)	--	1504
TAN	VR	47 (100)	49 (100)	4 (100)	1871
	SOL	--	24 (91)	76 (88)	1606

groups CON1, CON2, EAE, EAN, TAE and TAN respectively). The remaining percentages represented low values of SO fibers 14%, 8%, 4%, 3%, 8% and 4% respectively. The PAS staining showed a uniform depletion of glycogen stores in all fibers.

The SOL muscles demonstrated predominantly slow-twitch characteristics (Figure 4 and Table II) (68%, 80%, 79%, 79%, 82%, 76% and the remaining percentage being FOG fibers for group CON1, CON2, EAE, EAN, TAE and TAN respectively). Practically all fibers showed little staining intensity, indicating a severe glycogen depletion after the acute test.

4. Phosphorylase enzyme activity

Phosphorylase enzyme activity was measured in the chronic groups (which were sacrificed at least 48 hours after the performance test) and values are reported in micromoles per minute per gram of wet muscle tissue (μmoles/min/g).

The fast-twitch vastus lateralis muscles demonstrated higher enzyme activity than the slow-twitch soleus muscles (Figure 5). A one-way ANOVA showed a significant overall F-ratio in soleus muscle (Appendix D-6, c). However, only the initial control group mean showed a significant lower value (Appendix D-6, d) whereas age-matched control group (CON2) had similar values than trained groups indicating that training did not produce significant alterations.

5. Hexokinase enzyme activity

The enzymatic activity was measured at rest (chronic group

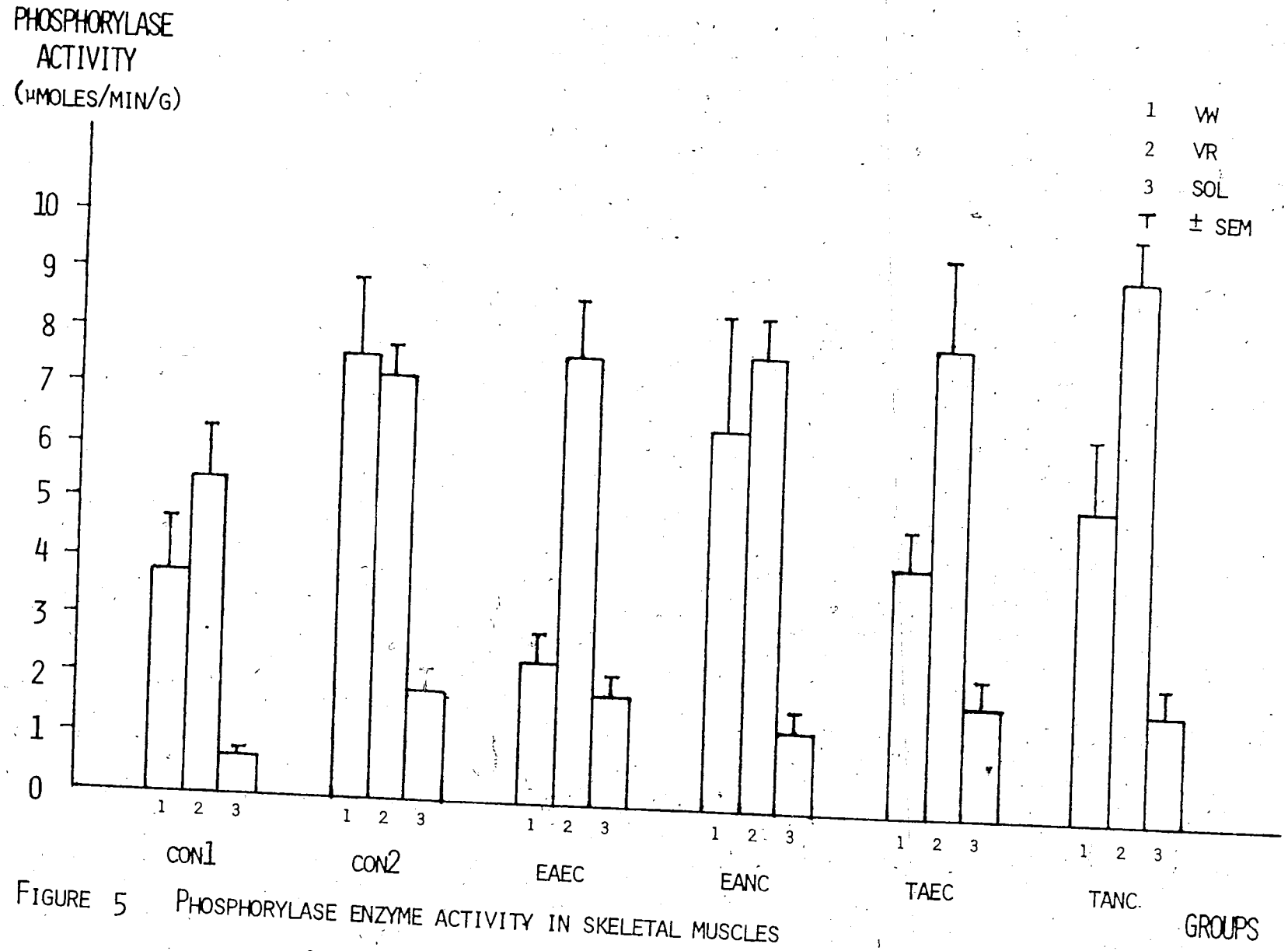


FIGURE 5 PHOSPHORYLASE ENZYME ACTIVITY IN SKELETAL MUSCLES

sacrificed at least two days after the performance test). The values are expressed as micromoles of substrate transformed per minute per gram of wet muscle tissue (μmoles/min/g).

Hexokinase activity showed a considerable ($p<.05$) 57% increase in the VW muscle of group TANC as compared to its age-matched control (CON2) (0.77 and 0.49 μmoles/min/g respectively) (Figure 6). Although not significant, the other groups showed a 16%, 20%, 20% increase (0.57, 0.59 and 0.59 μmoles/min/g) in groups EAEC, EANC and TAEC respectively as compared to group CON2 (Appendix D-7, a and b). Values of hexokinase activity for CON1 and CON2 are very similar (0.46 and 0.49 μmoles/min/g respectively).

Hexokinase enzyme activity in vastus lateralis red can be seen in Figure 6 (0.73, 0.80, 0.99, 0.95, 1.07 and 1.40 μmoles/min/g for groups CON1, CON2, EAEC, EANC, TAEC and TANC respectively). A one-way ANOVA summarized in Appendix D-7, c showed a significant difference between groups. A Scheffé test (Appendix D-7, d) was then applied to look at the difference between means. The results showed a significant difference between group TANC and CON1, CON2 and EANC revealing a 75% increase in hexokinase activity after the anaerobic training period for TANC as compared to CON2 and a 47% ($p<.05$) as compared to EANC.

Of the three skeletal muscles assayed, the hexokinase values registered for soleus muscle were the highest as reflected in group means (0.88, 1.20, 1.38, 1.36, 1.56 and 1.66 μmoles/min/g for groups CON1, CON2, EAEC, EANC, TAEC and TANC respectively) (Figure 6). No significant difference was found between the group means.

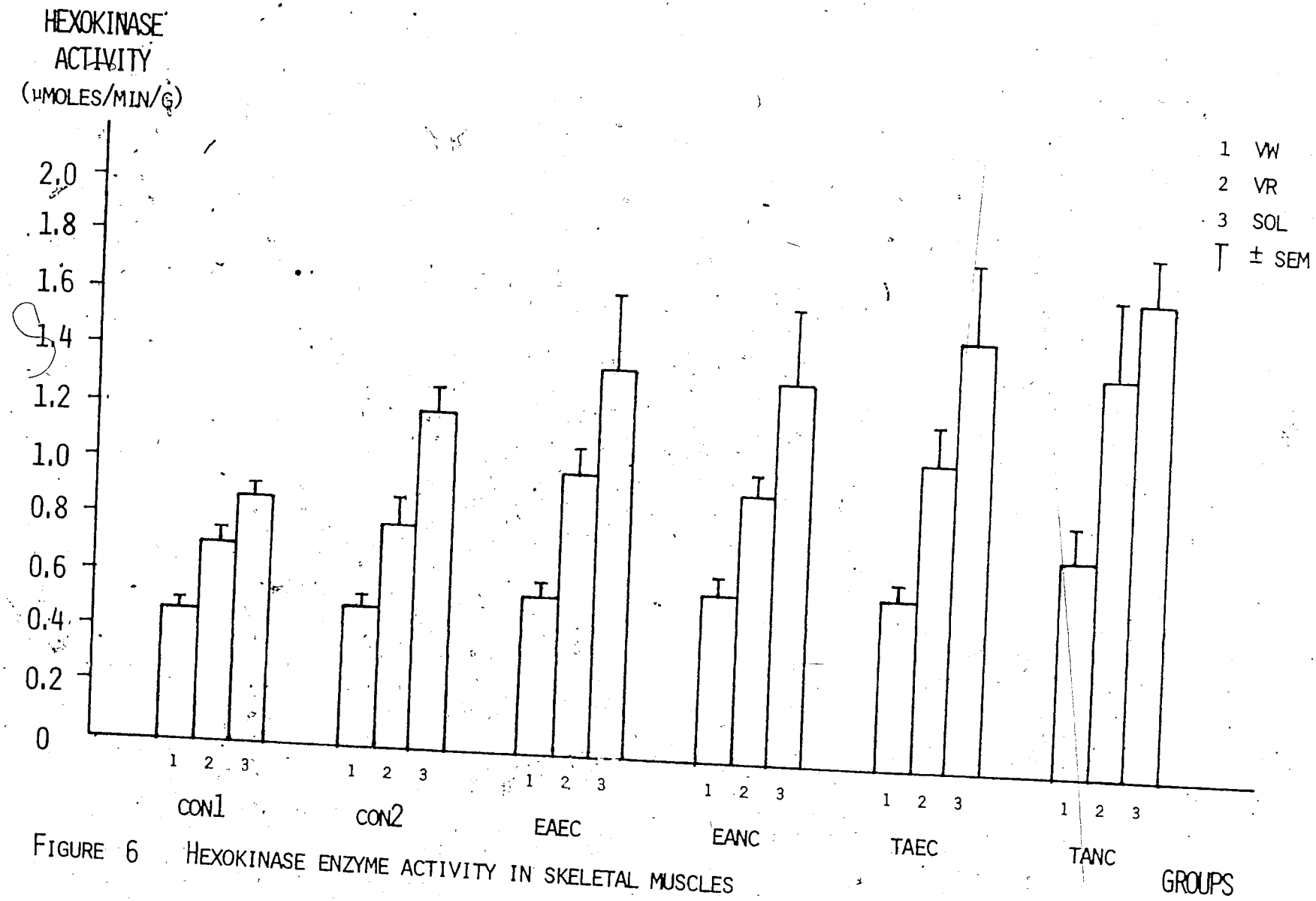


FIGURE 6 HEXOKINASE ENZYME ACTIVITY IN SKELETAL MUSCLES

CHAPTER V

DISCUSSION

1. Body weight

The steady body weight increase throughout the training period is shown in Figure 1. At the end of the experiment, trained rats weighed significantly less than the free-eating sedentary controls. This has been observed with guinea pigs trained by treadmill running (Barnard et al., 1970) and with rats trained for endurance (Huston et al., 1975, Muller, 1975, Baldwin et al., 1975), sprint training (Staudte et al., 1973, Houston and Green, 1975) or subjected to isometric training (Zika et al., 1973, Exner et al., 1973a and b).

Increased energy expenditure for the trained groups does not seem to be the only factor responsible for the body weight diminution in the present study. It has been suggested (Houston and Green, 1975) that an appetite-suppressing effect associated with vigorous exercise programs may combine with the increased energy expenditure of the training program resulting in lower body weights in these animals. Discrepancies in weight differences between different studies may result in various factors including age, sex, intensity, duration and type of training regimens. The body weights of the exercised groups (once a week) were between those of the trained and sedentary groups but not different ($p>.05$) than either. This would suggest that regardless of whether the decreased body weights are due to depressed

appetite or increased energy expenditure, the amount of exercise is a major determinant of body weight. Also, the use of exercised controls may be a more realistic comparison for body weight changes due to training.

No attempt was made in the present study to correlate muscle weights to body weights due to the subjective nature of the vastus lateralis muscles separation in their white and red portions.

2. Performance time

A 3.2-fold increase in anaerobic work time was observed in group TANC over EANC and a 2.4-fold increase in aerobic work time was seen in group TAEC over EAEC suggesting that the training regimen led the animals in groups TAEC and TANC to a substantial improvement in working times to exhaustion at their respective intensities as compared to their exercised counterparts in groups EAEC and EANC respectively.

However, a 3.7 times longer duration in performance time was depicted in Figure 2 between the anaerobically trained group (TANC) and the aerobically trained one (TAEC), even though their total physical work output was equivalent throughout their training sessions. This difference can be explained with the interpretation of Shepherd et al., 1976, which mentioned that the maximal oxygen uptake response in rats is elicited at a speed of 49.5 m/min. This indicates that the rats in group TAEC were trained continuously (40 m/min) at 90% of the workload eliciting maximal oxygen consumption whereas the rats in group TANC were trained intermittently (15 sec work, 30 sec rest) at 80 m/min for a total period of working time

two times less than group TAEC but at twice the velocity. The relatively high energy output required to run continuously at 40 m/min might have led the rats in group TAEC to a cessation of their run during the performance test due to the build up of acid metabolites (Wenger and Reed, 1976). Moreover, the anaerobic training program might have induced a greater potential to replenish the ATP and CP stores (Baldwin et al., 1975) during rest periods for group TANC or the intermittent nature of the work permitted replenishment of the phosphagens during recovery intervals and did not permit the build up of metabolites.

Although the physical work between the groups was equivalent (definition of terms, p. 4) no attempt was made to equate the metabolic work. However, the study of Essen et al., 1977, on humans, reported that substrate metabolism during equivalent continuous (55% $\dot{V}O_2$max) and intermittent (100% $\dot{V}O_2$ max, 15 sec work and 15 sec rest) exercise was similar even though work intensity was twice as high in intermittent as in continuous exercise.

In light of Cohen and Gans (1973) study on movement analysis, the gait of a white rat at 40 m/min is on the borderline between trot and canter/gallop suggesting that this transitional gait pattern might not have been an economical way of running as compared to a constant gait pattern seen at 80 m/min. White rats might be more suited to run at high speed for short period of time rather than continuously for longer period at relatively high speed (40 m/min) explaining their higher performance in the former condition. Unfortunately, no oxygen consumption tests were made in Cohen and Gans study.

3. Glycogen content

3.1 Muscle and liver glycogen

As judged by glycogen values in Figure 3, glycogen depletion from muscles involved in aerobic and anaerobic training appeared to be well represented by sampling vastus lateralis white, red and soleus muscles. The trained groups (TAEC or TAEA, TANC or TANA) did not demonstrate ($p>.05$) higher glycogen values as compared to the exercised groups (EAEC or EAEA, EANC or EANA) (11.0, 10.0, 14.3, 9.0 mg/g in VW; 9.3, 9.3, 12.6, 5.8 mg/g in VR; 12.0, 8.4, 11.4, 6.2 mg/g in SOL respectively) in the chronic situation and (1.9, 1.9, 2.6, 2.8 mg/g in VW; 2.0, 1.3, 2.4, 1.5 mg/g in VR; 2.3, 1.5, 1.7, 2.3 in SOL respectively) in the acute situation. This is somewhat comparable to the Baldwin et al., 1975, study on skeletal muscle glycogen depletion for trained (swimming and running) versus untrained (10.1, 9.8 mg/g in VW; 8.2, 8.1 mg/g in VR; 5.1, 4.7 mg/g in SOL respectively) in the resting condition and (5.6, 2.8 mg/g in VW; 5.1, 2.3 mg/g in VR; 2.8, 1.7 mg/g in SOL respectively) after a 45 minute running performance test at 24 m/min.

The findings in the present study on muscle glycogen depletion did not corroborate the conclusions of Baldwin et al., 1975, concerning the protective effect of training on muscle glycogen stores after a performance test since no difference in glycogen levels were found between trained and exercised groups after the acute test. Higher glycogen values in the trained animals as compared to the untrained animals were reported (Baldwin et al., 1975) after a 45 min of treadmill exercise (24 m/min) whereas in the present, a five minute acute

test brought the glycogen levels to relatively low values (Figure 3) in both the exercised and trained groups. The higher work intensity (40 m/min) performed in the present study during the acute test would necessitate a higher rate of energy production relying more on glycogen, the primary substrate for glycolysis (Wenger and Reed, 1976). Whereas, the lower work intensity in the Baldwin et al., 1975, study would favor lipid utilization, aerobic energy release and hence sparing of glycogen stores.

The glycogen levels in the chronic groups (Figure 3) may not be a reflection of the basal values for the acute groups because of the nature of the performance test administered to the chronic groups two days before sacrificing. The glycogen levels of the chronic groups in all fibers analyzed were expected to be totally depleted after the acute test to exhaustion. Two days may not have been sufficient to increase glycogen levels to significantly higher values in the exercised and trained groups over CON2 especially with an ordinary diet (Appendix F) based on protein rather than carbohydrate.

Although, when comparing trained groups (T_{10}, T_{30}, T_{60}, T_{120}) with sedentary controls, Fitts et al., 1975, reported a supercompensation effect in gastrocnemius muscle (10.4, 9.8, 10.4, 10.0 and 8.0 mg/g respectively) in all trained groups after a running. Baldwin et al., 1975 saw little or no glycogen supercompensation in the trained muscles as compared to sedentary control [illegible] 9.8 mg/g in VW; 8.2, 8.1 mg/g in VR; 5.1, 4.7 mg/g in SOL respectively). However, (Baldwin et al., 1975) the animals were sacrificed only eighteen hours after the last bout of exercise and not necessarily allowing for substantial glycogen repletion.

In the present study, the absence of different glycogen levels in the chronic situation between exercised and trained animals may be due to similar repletion rates but the trained animals may have shown higher glycogen levels if the sacrifice had been extended.

Liver glycogen did not show any appreciable depletion in the acute situation for the exercised and trained groups as compared to the sedentary control (CON2) indicating that the acute test might not have been long enough to bring about a significant decrease in liver glycogen stores (Figure 3). A substantial amount of time would be required to bring about hormonal changes which would initiate glucogenesis in the liver. Ahlborg et al., 1976 reported no change in glucagon after 40 min of exercise performed at 30% of $\dot{V}O_2$ max whereas Böttger et al., 1972, reported a significant rise in glucagon over the basal value only 10 min after the subjects reached the exhaustion point.

3.2 Glycogen depletion in muscle fibers

Comparative serial sections are shown in Plates Ia, Ib and Ic demonstrating glycogen depletion in different fiber types using ATPase, NADH diaphorase and PAS stains respectively. Fibers negatively stained in Plate Ic were considered glycogen depleted and were compared to Plates Ia and Ib in order to determine muscle fiber type depletion (FG, FOG or SO).

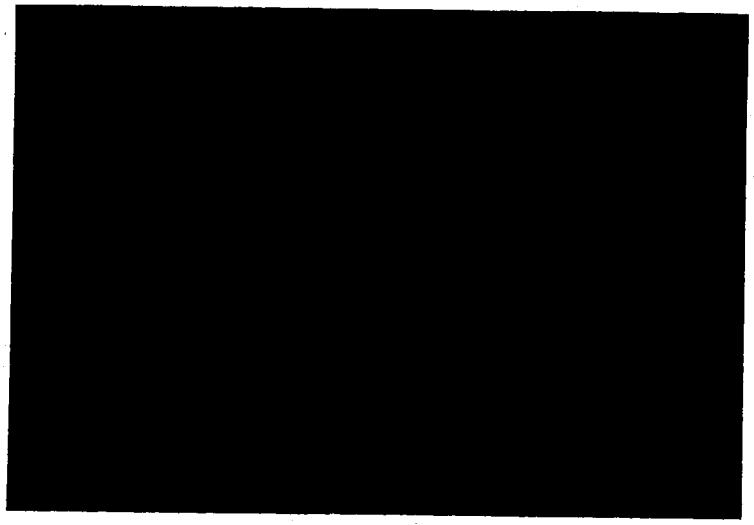
The subjective evaluation of PAS staining using an arbitrary scale limited somewhat the interpretation of the data and no quantitative estimation could be performed due to the staining saturation for carbohydrate concentration above 90 mM/Kg (Saltin, 1973). Hence

Plate I Micrographs showing serial sections of vastus lateralis red muscle stained for fiber types and glycogen content.

Plate Ia Myosin ATPase

Plate Ib NADH diaphorase

Plate Ic Periodic acid Schiff (PAS)



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it is only speculative when implying recruitment from glycogen depletion patterns.

In vastus lateralis red and soleus muscles, practically all fibers were glycogen depleted in all acute test groups (Table II); the few remaining glycogen stores were found mainly in fast-twitch fibers suggesting that the acute test performed by the rats was sufficiently strenuous to result in recruitment of most FG, FOG and SO fibers in these muscle.

The white vastus lateralis muscle showed a somewhat different glycogen depletion pattern as a result of the acute test (Table II). The more intense PAS staining in FG fibers suggests that these fibers did not participate in the exercise performed by each group to the same extent as the FOG fibers assuming the same initial levels existedin all fiber types. For an equivalent physical work output, groups TANA and TAEA demonstrated 36% and 45% depletion of glycogen stores in FOG fibers respectively. This small variation might be attributed to a greater use of carbohydrate in continuous work at approximately 90% $\dot{V}O_2$ max than in intermittent work at twice the intensity when relief periods permit oxidative replenishment of ATP and CP for the next work interval. However, in the non-trained groups (EAEA and EANA) the depletion pattern was the opposite. Greater depletion (65%) observed in the FOG fibers of the EANA group and only 47% in EAEA. This would imply that the exercised group EANA does not have the same capability as the TANA group to replenish the ATP and CP stores; it would have to rely more on carbohydrate stores in order to keep up with the energy demand. On the other hand, little information can be drawn from FG fiber recruitment as reflected by

equivalent percent glycogen depletion in all groups (Table II). Glycogen concentration in FG fibers might have been more elevated than FOG or SO fibers, therefore catabolizing similar amounts of glycogen at that speed and duration but the carbohydrate depletion was not detected because of the low saturation level of the PAS stain.

This trend can be related to studies done on relatively prolonged or intense work in humans (Gollnick et al., 1973, 1973a, 1974a, Costill et al., 1971, 1973) and in rats (Baldwin et al., 1973, 1973a, 1975, Armstrong et al., 1974) where the FG fibers, capable of handling higher tension, have been glycogen depleted when the work load exceeded the capacity of the FOG and SO fibers. Although not conclusive in the present investigation, fiber utilization and glycogen depletion patterns are more related to work intensity than duration when total physical work output is equivalent. The FG fibers appear to contribute to a greater extent to the exercise when the intensity of the work increases or when the oxidative fibers (FOG and SO) become glycogen depleted (Gollnick et al., 1973, Costill et al., 1973). Considering the larger oxidative capacity and blood supply of the FOG and SO fibers, it seems reasonable to have them the most easily activated resulting in a more economical use of the energy reserves of the muscle. Finally, the higher hexokinase activity in FOG and SO fibers as compared to FG fibers (Figure 6) would compensate for the preferential glycogen utilization especially between training sessions by augmenting the G-6-P pool in muscle (Piehl et al., 1974, Newsholme and Start, 1973).

The importance of carbohydrate sources at the onset of high intensity exercise has been emphasized extensively (Gollnick et al.,

1973, 1974a, Hermansen et al., 1967, Bergstrom and Hultman, 1967). As the exercise proceeds, more energy is derived from sources other than muscle carbohydrates such as blood glucose, liver glycogen, lactate and free fatty acid oxidation (Gollnick et al., 1974, Essen et al., 1977). It has been reported (Essen et al., 1977) that the contribution from fat to the energy requirement was similar in intermittent and continuous exercise when the total amount of work was almost identical. A very high rate of glycolysis would have occurred in the present study for the anaerobic groups if the same work load was performed continuously, but the rest periods might have permitted a more complete elimination of anaerobic by-products as those described by Wenger and Reed, 1976, or metabolic utilization of lactate (Belcastro and Bonen, 1975). This probably permitted half of group TANc to perform over 340 exercise bouts (over 85 min of work time) before reaching exhaustion. Also, their muscle cells probably derived more energy from carbon sources other than carbohydrates such as lactate and FFA, oxidizing them by the citric acid cycle during the relief periods in order to replenish the high energy phosphagens (Baldwin et al., 1975).

4. Enzyme activity

A brief summary (Table III) compares enzyme levels between different studies for sedentary control values. No further attempt will be made to discuss in great detail their differences. It is worthwhile though, to mention that a great deal of variability is found between authors because of specific factors including: species,

Table III Comparative enzyme activities in skeletal muscles of sedentary control animals

ENZYMES	SPECIES	MUSCLES	ENZYME ACTIVITIES[1] (μmoles/min/g)	AUTHORS
	rabbit	EDL	29.5	Bass et al., 1969
	rabbit	SOL	2.67	Bass et al., 1969
	man		8.5	Bass et al., 1969
	man		12.6	Russell et al., 1970
	rat		215 ±15	Baldwin et al., 1973
	rat		105 ±8.0	Baldwin et al., 1973
	rat		27 ±2.0	Baldwin et al., 1973
	man		15 ±2.0	Harris et al., 1976
	rat	GA	30.2 ±0.9	Huston et al., 1975
	rat	RF	11.5 ±1.5	Exner et al., 1973
Pase	rat	SOL	2.1 ±0.2	Exner et al., 1973
	rat	RF	24.0 ±3.0	Exner et al., 1973a
	rat	RF	30.2 ±2.8	Staudte et al., 1973
	rat	SOL	2.0 ±0.9	Staudte et al., 1973
	rat	GAR	71.8 ±3.1	Saubert IV et al., 1973
	rat	GAW	126.8 ±7.7	Saubert IV et al., 1973
	rat	VR	125.3 ±5.1	Saubert IV et al., 1973
	rat	SOL	12.2 ±0.1	Saubert IV et al., 1973
	rat	VW	7.6 ±1.5	Present study
	rat	VR	7.2 ±0.6	Present study
	rat	SOL	1.9 ±0.3	Present study
	rabbit	EDL	0.37	Bass et al., 1969
	rabbit	SOL	1.58	Bass et al., 1969
	rat	VW	0.58 ±0.04	Baldwin et al., 1973
	rat	VR	1.50 ±0.05	Baldwin et al., 1973
	rat	SOL	1.57 ±0.13	Baldwin et al., 1973
	g.p.	GA	0.49 ±0.02	Barnard and Peter, 1969
	man	RF	2.30 ±0.30	Bass et al., 1975
HK	rat	GA	1.13 ±0.05	Huston et al., 1975
	rat	RF	0.81 ±0.10	Exner et al., 1973
	rat	SOL	1.22 ±0.30	Exner et al., 1973
	rat	RF	1.00 ±0.09	Staudte et al., 1973
	rat	SOL	1.64 ±0.17	Staudte et al., 1973
	rat	VW	0.49 ±0.03	Present study
	rat	VR	0.80 ±0.09	Present study
	rat	SOL	1.20 ±0.09	Present study

1) Mean ±SEM

EDL: extensor digitorum longus, VW: vastus lateralis white, VR: vastus lateralis red, SOL: soleus, GA: gastrocnemius R(red) W(white), ET: erector trunci, RF: rectus femoris, g.p.: guinea pig.

sex, age, muscle and method used. However, further discussions will mention rate of change in enzyme activities following experimental conditions.

Phosphorylase enzyme activity seemed to follow a regular growth pattern throughout this investigation, however, only SOL muscle showed a significant higher ($p<.05$) phosphorylase activity in CON2 over CON1. The different exercise regimens tended to keep phosphorylase activity at the same level as the age-matched control values as can be seen by a non-significant difference (Appendix D-6) between group means in exercised and trained animals as compared to CON2. Although some studies (Huston et al., 1975, Exner et al., 1973, Saubert IV et al., 1973, Baldwin et al., 1973, 1973a, Staudte et al., 1973) reported small increases in phosphorylase in certain muscles, small decreases or no change have been found in the same study in other muscles after a training session. These discrepancies may be due to different relative contribution of the various muscles in work performance. No significant increase in phosphorylase activity was observed in this investigation for the muscles analyzed indicating that the normal levels of phosphorylase was adequate to meet the metabolic demands of the workloads without adaptation to higher levels of activity.

Hexokinase enzyme activity showed a more consistent pattern in all muscles studied. The 57% and 75% increases ($p<.05$) for group TANC over CON2 in HK activity in VW and VR respectively, are in agreement with Staudte et al., 1973, who reported a 47% increase in rectus femoris of rats after a three week sprint training program and also with Baldwin et al., 1973, where a twelve week endurance program led to a 170% and 30% increase in the level of hexokinase in VR and VW

respectively. TAEC group demonstrated slightly lower ($p>.05$) hexokinase activity as compared to TANC in all muscles studied which may suggest that the increase in hexokinase activity is more intensity-related rather than duration-related.

5. General discussion

Although the capability for increased glucose phosphorylation as indicated by hexokinase activity was increased with anaerobic training in selected muscles (VW and VR) this was not demonstrated by increased glycogen sparing in the acute exercise test. Since the glycogen levels 48 hours following the chronic test to exhaustion were used as initial glycogen levels prior to the acute test, the levels after the 48 hour interval may not have been a realistic estimate of the pre-acute test levels. This may have resulted in an underestimate of the glycogen utilization during the acute test. Also, the shortness of the acute test itself (5 min) due to the relatively high intensity in the aerobic group may not have been sufficient to reflect a glycogen sparing effect. In addition, the relatively high protein diet may have prevented glycogen stores from being fully replenished in only 48 hours.

When equivalent work loads were calculated, the work performed by the aerobic group was assigned as the training load for the anaerobic animals. Since the aerobic animals trained at 40 m/min continuously, this total work was not substantial because of the early onset of fatigue. The anaerobic group averaged 20 bouts per session when assigned the equivalent amount of work. This was only minimal

in terms of their capacity since the final performance test resulted in some animals completing over 340 bouts. If the running speed of the aerobic group had been lower (e.g. 30 m/min or less) both groups would have been overloaded to a greater extent and hence greater differences might have resulted between the trained and exercised groups. Also, the exercised groups may have received enough overload to achieve some training effect and hence result in the lack of differences.

When comparing the trained animals to sedentary controls, the TANC group alone showed significant changes and only in hexokinase in VW and VR muscles. However, when compared to exercised controls (EANC) only hexokinase in VR was elevated ($p<.05$). The exercised controls were not different ($p>.05$) from CON2 values on any dependent variable. However, the exercised animals were slightly higher than sedentary animals and hence resulted in a non-significant difference in hexokinase activity between anaerobically trained and exercised groups in VW muscle. This would suggest that exercised controls are a more realistic model for comparing training effects than the traditional sedentary age-matched animals whose enzyme, fuel and metabolite levels may be abnormally depressed due to confinement.

SUMMAI

Sixty-two male Wistar rats (110 g) were divided in five groups: sedentary control (CON), exercised aerobic (EAE), exercised anaerobic (EAN), trained aerobic (TAE) and trained anaerobic (TAN).

Following a two-week progressive pre-training session, the aerobically trained animals (EAE and TAE) and the anaerobically trained animals (EAN and TAN) were able to run continuously for 5 min at 40 m/min-15% grade and intermittently for 10 bouts (15 sec work and 30 sec rest) at 80 m/min-15% grade, respectively. The exercised animals ran only once a week and the trained rats were run twice a day, four days a week thereafter for eleven weeks. The running schedule allowed for a gradual training overload. Equivalent physical work output was assigned between exercised (EAE and EAN) and trained (TAE and TAN) groups during all running sessions in order to assess the relative contribution of intensity and duration in glycogen utilization and enzyme adaptation over a 3 month training program.

Hexokinase activity increased 57% ($p<.05$) in vastus lateralis white muscle for TANC over CON2; 75% ($p<.05$) and 47% ($p<.05$) in vastus lateralis red muscle for TANC over CON2 and EANC respectively. In an equivalent physical work output performed aerobically (40 m/min continuously), group TAEC demonstrated a slight increase in hexokinase activity (20%, 34% and 27% in VW, VR and SOL muscles respectively) but without reaching significance ($p>.05$) suggesting that hexokinase adaptation is intensity-related. Adaptation in total physical work

time was evaluated in a performance test to exhaustion which revealed a significantly higher running time for group TANC than group [illegible] but both groups reached a significant 3.2- and 2.4-fold increase in working times over EANC and EAEC respectively. Glycogen content in skeletal muscle and liver did not reveal a significant difference between groups in both chronic and acute situations.

CONCLUSIONS

Although there was no difference ($p>.05$) in body weight between the trained groups or between the trained and exercised animals, the sedentary controls were heavier ($p<.05$) than the trained groups at fifteen weeks of age.

Training led to biochemical adaptations in glucose phosphorylation following high intensity (anaerobic), short duration training as a result of an increase ($p<.05$) in hexokinase activity in selected skeletal muscles as compared to exercised and sedentary controls.

No differences ($p>.05$) in hexokinase or phosphorylase activity existed between the two different types of training regimens.

Work time to exhaustion was specific to the different training formats.

Skeletal muscle glycogen stores did not seem to be spared in any groups during a short acute test at 40 and 80 m/min.

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APPENDIX A

LIST AND SOURCE OF CHEMICALS

LIST OF CHEMICALS USED

CHEMICAL	SOURCE	
ATP (Na_2 salt) from equine muscle	SIGMA	A-3127
EDTA	FISHER	S-311
L-GLUCOSE	SIGMA	G-5500
G-1-P (K_2 salt) (Grade V)	SIGMA	G-6750
G-1-$P^{14}C$ (K_2 salt)	NEC	390
G-6-PDH (Type XI)	SIGMA	G-8898
GLYCOGEN (Type III) from rabbit liver	SIGMA	G-8876
LITHIUM BROMIDE	MCB	LX-320
MERCAPTOETHANOL	FISHER	4196
$MgCl_2$-$6H_2O$	SIGMA	M-0250
β-NADH (Na_2 salt)	SIGMA	N-8129
NADP (Na salt)	SIGMA	N-0505
SODIUM GLYCEROPHOSPHATE	FISHER	S-314
SODIUM FLUORIDE	FISHER	S-299
TRICHLOROACETIC ACID	FISHER	A-322
TRIZMA-HCl	SIGMA	T-3253

APPENDIX B

BIOCHEMICAL ASSAYS

Appendix B-1

α-glucan phosphorylase (Pase) (EC 2.4.1.1)

(α-1,4-glucan: orthophosphate glucosyltransferase)

Reference: Russell J.C., Tougas D., Taylor A.W. Rapid assay for glycogen-cycle enzymes in small samples of muscle. Clinical Chemistry 16:900-902, 1970.

Principle: G-1-P^{14}C + glycogen $\xrightarrow[\text{Pase}]{}$ glycogen^{14}C

Glucose-1-phosphate^{14}C incubated with glyco[illegible] in the presence of phosphorylase will [illegible]erate readily in the reverse reaction if sufficien[illegible]ucose-1-[illegible]hosphate is present, yielding glycogen. Radioa[illegible]ivity is incorporated into the glycogen primer and the rate of the enzymatic reaction is reported in terms of μmoles/min/g.

Reagents:

1. Sodium glycerophosphate
2. EDTA
3. Sodium fluoride
4. Mercaptoethanol
5. Glycogen
6. Lithium Bromide
7. Trichloroacetic acid
8. G-1-P
9. G-1-P^{14}C (50 μCi, uniformly labeled)

Procedure:

1. Buffer solution: prepare desired volume containing:
   - Sodium glycerophosphate 20 mmoles
   - EDTA 1 mmole
   - Sodium fluoride 100 mmole
   - Mercaptoethanol 20 mmoles pH: 6.1

2. Substrate solution[1]: using buffer solution as solvent, add:
   - Glycogen 20 g/liter
   - G-1-P + G-1-P^{14}C 32 mmoles

3. Stop solution: each ml contains:
   - Trichloroacetic acid 60 mg
   - Lithium bromide 2 mg
   - Glycogen 1 mg

4. Incubate tubes containing 0.1 ml of substrate solution together with the muscle extract samples for 15 min at 30°C

5. 0.1 ml of the muscle extract sample was added to the substrate tube (12 x 75 mm) and shaken. Reaction was allowed to proceed 5 minutes. (30°C)

6. 1.0 ml of ice cold stop solution was added at the end of the reaction

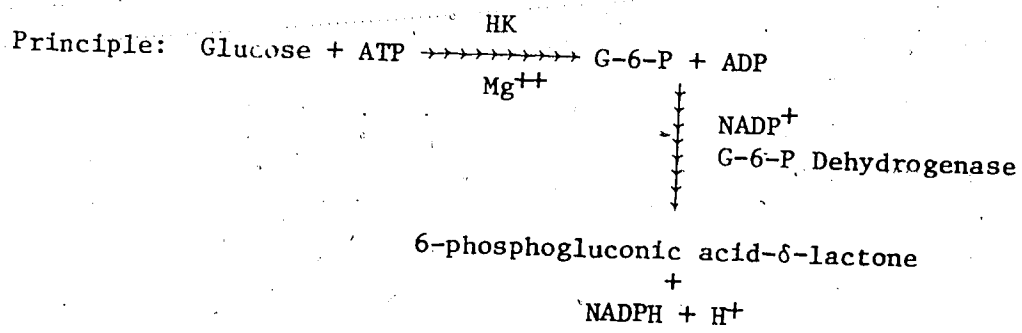
1) 0.1 ml of substrate solution was pipetted in small test tubes (12 x 75mm) and kept frozen.

Appendix B-2

Hexokinase (HK) (EC 2.7.1.1)
(ATP:D-Hexose 6-phosphotransferase)

Modified from: Joshi M.D., Jagannathan V. in Methods in Enzymology, Ed. S.P. Colowick, N.O. Kaplan, Vol IX, p. 371, 196

Principle:



The formation of NADPH can be readily monitored at 340 nm providing an excess ATP and $NADP^+$ because the equilibrium constant values of the hexokinase reaction and the glucose-6-phosphate dehydrogenase reaction lie very far to the right.

Reagents:

1.	Glucose	0.15 M
2.	$MgCl_2$-$6H_2O$	0.20 M
3.	EDTA	0.0001 M
4.	Tris-HCl buffer	0.20 M pH 7.0
5.	NADP	0.0039 M
6.	G-6-P Dehydrogenase[1]	2 units/ml
7.	ATP (Na salt)	0.45 M pH 7.0

1) Should have negligible amount of G-6-Phosphatase, NADPase, NADPH oxidase, Hexokinase, Glucose-NADP reductase, and 6-phospho-gluconic dehydrogenase.

Procedure:

1. Stock solution[1]:

   Mix together 7.5 ml. of the reagents 1, 2, 3 and 10 ml of reagent 4
   Once ready to start enzyme assays, add:
   7.5 ml of reagent 5

2. Pipet 1.7 ml of stock solution to a 1 cm cuvette with 0.3 ml of reagent 6,
   and 0.1 ml of homogenate solution (sample cuvette)
   and 0.1 ml of reagent 4 (reference cuvette)

3. Incubate both cuvettes for 15 min at 37°C

4. Add 0.1 ml of reagent 7 to both cuvettes and record rate of formation of NADPH at 340 nm against time (cell holder kept at 37°C).

1) prepared for 20 assays and kept cold

APPENDIX C

RAW DATA

Appendix C-1. Raw data for BODY WEIGHTS

Groups	Body weights (g)				
	1	2	3	4	5
CON2	110	155	242	334	371
	128	168	273	352	395
	147	236	340	432	468
	148	209	297	409	455
	135	191	272	383	417
	146	216	320	410	477
	132	175	266	354	384
EAE	138	181	263	352	381
	132	186	259	346	376
	112	152	250	355	396
	123	191	285	366	397
	141	195	279	365	397
	122	189	291	294	390
	15	219	291	365	408
	130	[illegible]	[illegible]	349	364
	145	[illegible]	272	367	408
	144	198	275	340	366
	154	220	307	357	393
	144	202	297	399	423
EAN	133	215	292	364	398
	153	183	250	338	357
	124	153	252	338	374
	144	195	259	360	408
	137	200	295	393	435
	126	174	255	343	361
	145	204	288	370	407
	153	218	272	347	370
	139	203	289	371	399
	132	180	257	365	402
	136	173	[illegible]	326	360
	127	159	241	327	357
TAE	121	163	237	295	321
	156	219	296	338	344
	153	154	214	377	415
	128	178	243	298	314
	154	211	290	360	393
	146	193	261	347	386
	133	175	250	315	351
	139	183	263	347	399
	149	212	266	357	374
	150	198	284	326	354
	130	173	258	331	367
	147	195	270	341	372

Appendix C-1 (cont'd) Raw data for BODY WEIGHTS

Groups	Body weights (g)				
	1	2	3	4	5
TAN	148	196	265	339	393
	145	199	284	385	422
	132	189	262	352	381
	127	174	235	310	351
	141	192	277	335	384
	144	201	279	355	394
	114	153	233	315	341
	159	154	261	334	350
	133	192	284	381	403
	160	212	277	357	385
	142	178	270	348	385
	145	175	247	318	360

1) November 30 (week 1 in lab.)
2) December 12 (week 3 in lab.)
3) January 2 (week 6 in lab.)
4) February 7 (week 11 in lab.)
5) Sacrificing time

Appendix C-2 Raw data for GLYCOGEN VALUES (mg/g)

Groups	Glycogen values (mg/g)							
	VWC	VWA	VRC	VRA	SOC	SOA	LIC	LIA
CON1	10.80	10.80	2.17	2.17	5.25	5.25	-	-
	1.56	1.56	6.49	6.49	8.49	8.49	-	-
	15.11	15.11	14.19	14.19	11.72	11.72	-	-
	10.80	10.80	14.96	14.96	21.12	21.12	-	-
	16.96	16.96	13.11	13.11	18.19	18.19	-	-
	17.58	17.58	14.80	14.80	11.47	11.47	-	-
	2.79	2.79	3.10	3.10	4.02	4.02	-	-
CON2	5.25	5.25	5.56	5.56	4.95	4.95	22.13	22.13
	5.41	5.41	7.26	7.26	5.25	5.25	28.73	28.73
	4.64	4.64	5.10	5.10	4.02	4.02	18.57	18.57
	8.64	8.64	5.87	5.87	6.49	6.49	11.97	11.97
	6.49	6.49	7.41	7.41	7.10	7.10	45.87	45.87
	3.90	3.90	3.28	3.28	4.22	4.22	12.80	12.80
	3.10	3.10	3.41	3.41	5.10	5.10	6.21	6.21
EAE	14.65	2.89	14.96	2.18	13.73	1.50	31.62	16.37
	11.57	1.63	12.03	2.25	10.95	0.99	31.31	32.85
	19.27	2.51	17.73	0.77	16.34	1.56	59.78	36.61
	8.18	3.79	6.95	2.08	6.18	2.38	29.30	28.64
	19.12	2.79	13.42	1.12	6.18	2.04	6.95	24.40
	12.96	2.12	10.69	6.07	15.27	1.73	25.25	16.29
EAN	7.10	3.13	5.10	1.26	4.28	1.91	11.97	9.38
	8.95	4.12	9.57	1.24	9.10	2.02	46.13	24.98
	14.65	2.00	2.94	1.56	1.40	2.28	11.68	33.32
	6.64	2.70	4.18	1.65	7.56	2.65	56.41	33.21
	8.33	3.19	4.79	1.89	7.41	2.22	56.41	22.22
	8.33	1.70	8.33	1.32	7.56	2.54	20.27	19.61
TAE	17.73	2.70	11.11	2.81	14.03	3.97	38.52	7.05
	5.79	3.21	4.84	1.05	13.26	1.60	36.65	28.67
	8.95	1.13	10.03	2.22	9.41	2.01	41.72	44.86
	7.72	1.04	7.41	1.94	9.26	1.99	18.03	13.52
	9.57	1.23	9.87	2.41	15.11	1.74	41.24	7.36
	16.19	2.03	12.18	1.61	10.64	2.29	21.21	41.40
TAN	8.80	1.09	8.03	1.13	8.80	1.12	22.09	18.23
	10.80	1.29	12.80	0.97	6.79	1.94	26.38	26.81
	7.72	1.22	6.95	1.32	8.64	1.36	56.20	7.79
	19.42	1.23	16.65	1.32	17.73	1.64	37.27	32.88
	7.72	1.36	7.56	1.23	4.33	1.26	42.50	49.21
	5.47	5.42	3.90	1.71	3.90	1.95	15.33	25.94

C: Chronic, A: Acute, LI: Liver.

Appendix C-3 Raw data for PERFORMANCE TEST

Groups	Performance time (min:sec)
EAEC	8:07
	10:25
	4:59
	5:30
	7:46
	8:13
EANC	17:15
	15:45
	27:45
	28:15
	16:00
TAEC	6:23
	6:57
	11:58
	20:09
	42:00
	22:16
TANC	27:00
	58:00
	85:45
	60:00
	87:15
	87:00

Appendix C-4 Raw data for PHOSPHORYLASE ACTIVITY (μmoles/min/g)

Groups	Phosphorylase (μmoles/min/g)		
	VR	VR	SOL
CON1	9.-	1.59	0.25
	1.99	9.87	0.80
	2.37	6.82	0.33
	1.63	8.21	0.59
	2.14	4.88	1.02
	5.93	3.76	0.47
	3.46	2.96	0.58
CON2	2.53	4.75	0.96
	5.86	8.63	1.15
	11.48	4.49	1.54
	8.20	8.69	3.19
	6.17	6.05	1.26
	4.65	8.14	2.60
	14.24	8.29	2.24
EAEC	2.32	5.78	2.15
	2.67	10.75	1.90
	4.15	8.14	1.34
	2.12	9.45	2.06
	1.38	7.21	1.26
	1.82	5.07	2.58
EANC	9.88	7.67	1.06
	14.50	8.81	1.52
	3.81	7.06	1.74
	5.90	9.64	1.30
	3.18	7.61	2.24
	1.56	5.88	0.68
TAEC	3.81	4.66	1.27
	5.31	8.01	2.75
	3.89	5.58	2.65
	5.02	6.45	1.36
	5.70	12.45	1.03
	1.38	10.78	2.19
TANC	6.40	6.62	1.46
	10.14	10.87	2.33
	3.21	8.91	1.70
	3.39	9.26	2.00
	2.62	11.25	1.17
	6.53	8.66	2.12

Appendix C-5 Raw data for HEXOKINASE ACTIVITY (μmoles/min/g)

Groups	Hexokinase (μmoles/min/g)		
	VW	VR	SOL
CON1	0.45	0.69	0.91
	0.45	0.57	0.65
	0.46	1.13	1.18
	0.47	0.69	0.91
	0.45	0.68	0.95
	0.46	0.69	0.88
	0.47	0.68	0.70
CON2	0.45	1.14	1.38
	0.69	0.90	1.12
	0.46	0.91	0.90
	0.46	0.90	1.61
	0.46	0.45	1.11
	0.46	0.57	1.14
	0.46	0.68	1.14
EAE	0.68	0.91	1.15
	0.45	0.68	0.91
	0.45	1.14	1.37
	0.68	1.13	1.39
	0.45	0.91	1.83
	0.68	1.13	1.62
EANC	0.68	0.91	0.89
	0.45	0.68	1.12
	0.45	0.91	1.60
	0.57	1.14	1.13
	0.69	1.13	1.82
	0.68	0.91	1.56
TAEC	0.68	1.13	1.38
	0.56	1.33	1.38
	0.68	1.14	1.35
	0.46	0.92	1.37
	0.45	0.76	1.37
	0.68	1.13	2.32
TANC	0.57	1.13	1.62
	0.69	1.59	1.63
	1.14	1.83	2.01
	0.76	1.37	1.61
	0.68	1.32	1.71
	0.75	1.14	1.35

APPENDIX D

SUMMARY OF ANALYSES OF VARIANCE AND SCHEFFE MULTIPLE COMPARISONS OF MEANS

Appendix D-1, a Summary of the analysis of variance for BODY WEIGHTS (Feb. 7) in sedentary, exercised and trained rats

Source of variation	DF	SS	MS	F	P
GROUPS	4	10217	2554	4.00	.007
ERROR	50	31952	639		

Homogeneity of variance $\chi^2=3.03$ P=.55

Appendix D-1, b Scheffé multiple comparison of means

Probability matrix

	CON	EAE	EAN	TAE	TAN
CON	-	.2828	.2460	.0109	.0552
EAE		-	1.00	.5244	.9029
EAN			-	.5[illegible]	.9327
TAE				-	.9606
TAN					-

Appendix D-1, c Summary of the analysis of variance for BODY WEIGHTS (sacrifice time) of sedentary, exercised and trained rats

Source of variation	DF	SS	MS	F	P
GROUPS	4	15892	3973	5.18	.001
ERROR	50	38366	767		

Homogeneity of variance $\chi^2=7.12$ P=.13

Appendix D-1. d Scheffé multiple comparison of means

Probability matrix

	CON	EAE	EAN	TAE	TAN
CON	-	.2144	.0889	.0022	.0316
EAE		-	.9891	.2842	.8757
EAN			-	.5670	.9897
TAE				-	.8445
TAN					-

Appendix D-2, a Summary of the analysis of variance for GLYCOGEN level in VW muscle of sedentary, exercised and trained rats in the CHRONIC situation

Source of variation	DF	SS	MS	F	P
GROUPS	5	273	55	2.45	.06
ERROR	32	713	22		

Homogeneity of variance χ^2=10.5 P=.06

Appendix D-2, b Summary of the analysis of variance for GLYCOGEN level in VW muscle of sedentary, exercised and trained rats in the ACUTE situation

Source of variation	DF	SS	MS	F	P
GROUPS	5	401	80	8.64	<.001
ERROR	32	297	9		

Homogeneity of variance χ^2=38.7 P<.01

Appendix D-2, c Scheffé multiple comparison of means

Probability matrix

	CON1	CON2	EAEA	EANA	TAEA	TANA
CON1	-	.0743	.0027	.0035	.0009	.0010
CON2		-	.7626	.8105	.5366	.5514
EAEA			-	1.00	.9993	.9995
EANA				-	.9980	.9984
TAEA					-	1.00
TANA						-

Appendix D-3, a Summary of the analysis of variance for GLYCOGEN level in VR muscle of sedentary, exercised and trained rats in the CHRONIC situation

Source of variation	DF	SS	MS	F	P
GROUPS	5	229	46	3.22	.02
ERROR	32	456	14		

Homogeneity of variance $\chi^2=9.7$ P=.08

Appendix D-3, b Summary of the analysis of variance for GLYCOGEN level in VR muscle of sedentary, exercised and trained rats in the ACUTE situation

Source of variation	DF	SS	MS	F	P
GROUPS	5	373	75	10.26	<.001
ERROR	32	232	7		

Appendix D-3, c Scheffé multiple comparison of means

Probability matrix

	CON1	CON2	EAEA	EANA	TAEA	TANA
CON1	-	.1252	.0050	.0004	.0010	.0003
CON2		-	.5630	.2618	.4160	.2121
EAEA			-	.9959	.9999	.9897
EANA				-	.9998	1.00
TAEA					-	.9988
TANA						-

Appendix D-4, a Summary of the analysis of variance for GLYCOGEN level in SOL muscle of sedentary, exercised and trained rats in the CHRONIC situation

Source of variation	DF	SS	MS	F	P
GROUPS	5	241	48	2.47	.05
ERROR	32	624	19		

Homogeneity of variance $\chi^2=17.7$ P=.003

Appendix D-4, b Summary of the analysis of variance for GLYCOGEN level in SOL muscle of sedentary, exercised and trained rats in the ACUTE situation

Source of variation	DF	SS	MS	F	P
GROUPS	5	502	100	12.61	<.001
ERROR	32	255	8		

Homogeneity of variance $\chi^2=69.4$ P<.001

Appendix D-4, c Scheffé multiple comparison of means

Probability matrix

	CON1	CON2	EAEA	EANA	TAEA	TANA
CON1	-	.0155	.0001	.0002	.0002	.0001
CON2		-	.4040	.5949	.5938	.3560
EAEA			-	.9997	.9997	1.00
EANA				-	1.00	.9990
TAEA					-	.9990
TANA						-

Appendix D-5, a . Summary of the analysis of variance for GLYCOGEN level in LIVER of sedentary, exercised and trained rats in the CHRONIC situation

Source of variation	DF	SS	MS	F	P
GROUPS	4	820	205	.90	.48
ERROR	26	5929	228		

Homogeneity of variance $\chi^2=1.7$ P=.79

Appendix D-5, b Summary of the analysis of variance for GLYCOGEN level in LIVER of sedentary, exercised and trained rats in the ACUTE situation

Source of variation	DF	SS	MS	F	P
GROUPS	4	136	34	0.21	.93
ERROR	26	4226	162		

Homogeneity of variance $\chi^2=3.1$ P=.54

Appendix D-6, a Summary of the analysis of variance for PHOSPHORYLASE activity in VW muscle of sedentary, exercised and trained rats

Source of variation	DF	SS	MS	F	P
GROUPS	5	114	23	2.25	.07
ERROR	32	325	10		

Homogeneity of variance $\chi^2=$ .3 P=.02

Appendix D-6, b Summary of the analysis of variance for PHOSPHORYLASE activity in VR muscle of sedentary, exercised and trained rats

Source of variation	DF	SS	MS	F	P
GROUPS	5	52	10	2.01	.10
ERROR	32	163	5		

Homogeneity of variance $\chi^2=5.6$ P=.35

Appendix D-6, c Summary of the analysis of variance for PHOSPHORYLASE activity in SOL muscle of sedentary, exercised and trained rats

Source of variation	DF	SS	MS	F	P
GROUPS	5	9	1.8	5.05	<.001
ERROR	32	11	0.4		

Homogeneity of variance χ^2=7.8 P.17

Appendix D-6, d Scheffé multiple comparison of means

Probability matrix

	CON1	CON2	EAEC	EANC	TAEC	TANC
CON1	-	.0200	.0228	.3352	.0235	.0393
CON2		-	1.00	.8601	1.00	1.00
EAEC			-	.8439	1.00	1.00
EANC				-	.8491	.9230
TAEC					-	1.00
TANC						-

Appendix D-7, a Summary of the analysis of variance for HEXOKINASE activity in VW muscle of sedentary, exercised and trained rats

Source of variation	DF	SS	MS	F	P
GROUPS	5	0.37	0.07	5.37	.001
ERROR	32	0.44	0.01		

Homogeneity of variance $\chi^2=29.9$ $P<.001$

Appendix D-7, b Scheffé multiple compariso of means

Probability matrix

	CON1	CON2	EAEC	EANC	TAEC	TANC
CON1	-	.9988	.7429	.5862	.5892	.0034
CON2		-	.9160	.8093	.8117	.0099
EAEC			-	.9999	.9999	.1502
EANC				-	1.00	.2405
TAEC					-	.2386
TANC						-

Appendix D-7, c Summary of the analysis of variance for HEXOKINASE activity in VR muscle of sedentary, exercised and trained rats

Source of variation	DF	SS	MS	F	P
GROUPS	5	1.78	.36	8.06	<.001
ERROR	32	1.42	.04		

Homogeneity of variance χ^2=1.63 P=.90

Appendix D-7, d Scheffé multiple comparison of means

Probability matrix

	CON1	CON2	EAEC	EANC	TAEC	TANC
CON1	-	.9968	.4645	.6457	.1712	.0003
CON2		-	.7534	.8908	.3844	.0011
EAEC			-	.9998	.9926	.0655
EANC				-	.9581	.0333
TAEC					-	.2219
TANC						-

Appendix D-7, e Summary of the analysis of variance for HEXOKINASE activity in SOL muscle of sedentary, exercised and trained rats

Source of variation	DF	SS	MS	F	P
GROUPS	5	2.4	.48	5.81	<.001
ERROR	32	2.7	.08		

Homogeneity of variance χ^2=5.01 P=.41

Appendix D-7, f Scheffé multiple comparison of means

Probability matrix

	CON1	CON2	EAEC	EANC	TAEC	TANC
CON1	-	.5222	.1164	.1522	.0177	.0026
CON2		-	.9357	.9649	.5366	.1815
EAEC			-	1.00	.9765	.7333
EANC				-	.9547	.6585
TAEC					-	.9864
TANC						-

APPENDIX E

TRAINING PROGRESSION

Appendix E Training progression

Day	Time	Group	Speed m/min	Grade %	Rep	time on min:sec	time off min:sec	Interval[1]	Total on	Total off
1	AM	AE	10	5	4	0:30	0:30	–	2:00	2:00
		AN	10	5	4	0:30	0:30	–	2:00	2:00
2	AM	AE	15	10	3	0:30	0:30	–	1:30	1:30
		AN	15	10	3	0:30	0:30	–	1:30	1:30
3	AM	AE	20	15	4	0:30	0:30	–	2:00	2:00
		AN	20	15	4	0:30	0:30	–	2:00	2:00
6	AM	AE	20	15	4	1:00	1:00	–	4:00	4:00
		AN	30	15	1	1:00	1:00	6	2:30	3:00
7	AM	AE	20	15	4	1:30	1:00	–	6:00	4:00
		AN	40	15	1	1:00	1:00	6	2:30	3:00
9	AM	AE	30	15	3	1:30	1:30	–	4:00	4:00
		AN	60	15	2	0:30	1:30	6	2:30	5:00
	PM	AE	30	15	3	1:30	1:30	–	4:00	4:00
		AN	60	15	2	0:30	1:30	6	2:30	5:00
10	AM	AE	30	15	4	1:30	1:00	–	6:00	3:00
		AN	60	15	3	0:30	1:30	6	3:00	5:00
	PM	AE	30	15	4	1:30	1:00	–	6:00	3:00
		AN	60	15	3	0:30	1:30	6	3:00	5:00
13	AM	AE	30	15	1	4:30	--	–	4:30	--
		AN	60	15	–	--	--	9	2:15	3:00
	PM	AE	30	15	1	4:30	--	–	4:30	--
		AN	60	15	–	--	--	9	2:15	3:00
14	AM	AE	40	15	1	3:00	--	–	3:00	--
		AN	70	15	–	--	--	6	1:30	2:00
	PM	AE	40	15	1	3:00	--	–	3:00	--
		AN	70	15	–	--	--	6	1:30	2:00
16	AM	AE	40	15	1	3:00	--	–	3:00	--
		AN	70	15	–	--	--	9	1:30	3:00
	PM	AE	40	15	1	3:00	--	–	3:00	--
		AN	70	15	–	--	--	9	1:30	3:00
17	AM	AE	40	15	1	5:00	--	–	5:00	--
		AN	80	15	–	--	--	10	2:30	4:30
	PM	AE	40	15	1	5:00	--	–	5:00	--
		AN	80	15	–	--	--	10	2:30	4:30

[1]: intervals of 15 sec on and 20 sec off. Rep: Repetitions

APPENDIX F

LABORATORY CHOW INGREDIENTS

PURINA

LABORATORY CHOW

GUARANTEED ANALYSIS

Crude protein not less than	23.0%
Crude fat not less than	4.5%
Crude fiber not more than	6.0%
Ash not more than	8.0%
Added minerals not more than	2.5%

INGREDIENTS

Ground extruded corn, soybean meal, ground oat groats, dried beet pulp, wheat germ meal, fish meal, dehydrated alfalfa meal, dried milk products, cane molasses, meat and bone meal, brewers'dried yeast, wheat middlings, animal fat preserved with BHA, calcium carbonate, dicalcium phosphate, salt, calcium iodate, animal liver meal, vitamin B_{12} supplement, methionine hydroxy analogue calcium, calcium pantothenate, choline chloride, folic acid, thiamin, niacin, pyridoxine, ferrous sulfate, vitamin A supplement, vitamin E supplement, iron sulfate, iron oxide, manganous oxide, copper oxide, zinc oxide.